Government of Pakistan
Pakistan Environmental Protection Agency
(Ministry of Climate Change & Environmental Coordination)

PAKISTAN BIOSAFETY GUIDELINES

(February 2024)

Related Reference: Pakistan Biosafety Rules, 2005 (amendment 2024)
S.R.O. 336(I)/2005 S.R.O 45(I)/2024

Note: The Pakistan Biosafety Rules, 2005 will prevail in case of any contradiction or omission in these Guidelines

EXECUTIVE SUMMARY

1. Recognizing the revolutionary economic potential of the new biotechnology in agriculture, health, industry, environment and energy sectors; and appreciating the concerns that mixing of genes from unrelated organisms might create natural imbalance that is not yet adequately understood; and considering the fears that manipulated genes or products thereof if allowed to move around freely in nature, may pose potential hazards; and realizing the apprehensions that certain transgenic organisms may be harmful or become harmful to economic plants, animals and human being; and discharging the obligations of the Convention of Biological Diversity (CBD) and Cartagena Protocols to which Pakistan is a signatory; the Minister of Environment, constituted a National Biosafety Expert Committee to deliberate on these issues to regulate the safe release of Genetically Modified Organisms (GMOs) and products thereof.

2. Consequent upon extensive deliberations/consultation with all the stakeholders, the national committee concluded that there is a national need to develop biosafety guidelines to control laboratory research, field studies and commercial release of GMOs and products thereof, as have been done by all the developed countries and many developing countries.

3. Accordingly, a set of National Biosafety Guidelines have been developed through a national forum participated by all the stakeholders including the academic institutions, R&D organization, industry, non-government organizations and human right societies.

4. These guidelines have been prepared keeping in view the guidelines prepared by UNIDO, FAO, WHO, UNEP, and all the developed and developing countries with modification to suit our unique and specific socio-economic and geographic environment.

5. The objective of these guidelines is to prevent unintentional negligence leading to misuse and irresponsibility by laboratory workers/researchers as well as the end-users.

6. To define the bounds guidelines/regulatory jurisdiction of these guidelines, biotechnology has been defined as processes using living organisms or parts thereof to make or modify products; and to improve plants, animals, or microorganisms for specific uses. “Recombinant DNA” has been defined as molecules developed outside living cells by joining natural or synthetic DNA segments to DNA that can replicate in a living cell and those DNA molecules that result from the replication of such DNA.

7. For the purposes of these guidelines, regulated material includes all genetically modified materials (DNA & RNA preparations, viroids, viruses, cells and organisms, modified or constructed through genetic engineering), derivatives thereof and wastes or by-products of genetic engineering practices (containing viable organisms or otherwise).

8. The scope of these guidelines embraces all works related to gene manipulation employing recombinant DNA technology for all purposes including the development of transgenic plants, animals and microorganisms; production of vaccines; industrial manufacturing of Genetically Modified Organisms and products thereof, and their release into the environment for field trials as well as forcommercial uses.

9. The Guidelines consist of two parts; the first part relates to regulated work in laboratory research and field trials; and the second part deals with procedures for approvals which must be obtained to deregulate the regulated materials to allow their free movement and commercial uses.

10. All regulated laboratory research works are classified into (a) Minimal risk, (b) Low risk, (c) Considerable level of risk and laboratory containment conditions are accordingly prescribed. For regulated fieldwork, comprehensive containment conditions have been prescribed separately for genetically modified microorganisms, plants and animals.

11. The mechanism of monitoring and implementation of the proposed guidelines is built on three tiers as specified in the Biosafety Rules, 2005, namely a National Biosafety Committee (NBC); a Technical Advisory Committee (TAC); and Institutional Biosafety Committees (IBCs) at the institutional levels. The NBC headed by the Secretary, Ministry of Climate Change & Environmental Coordination will be responsible to oversee all laboratory work, field trials and allow commercial releases of GMOs and their products including Import for direct use as Food or Feed, or for Processing.

12. Enforcement of various clauses of the National Biosafety Guidelines will be administered by the three monitoring implementation bodies, as per legal authority under Clause 7(g) of the Pakistan Environment Protection Act 1997.

13. The IBC may recommend to NBC for awarding exempt status for Laboratory work/field work with genetically modified organisms, if there is sufficient information/grounds available to consider the work as having no risk and NBC may consider for formal approval.

14. Permission for deregulation granted by the NBC can be withdrawn if sufficient technical data/evidence becomes available after the approval, which warrants its deregulation.

15. To standardize processing, separate format for application/proposal for obtaining permission to undertake laboratory genetic manipulation work, field trial, import for direct use as Food or Feed, or for Processing and commercialization have been

designed.

16. A separate application format has also been designed for the movement of regulated materials and/or exempt status.

17. Instructions for the preparation of applications as well as for its assessment have also been prescribed to streamline and standardize the procedures for assessment and evaluation.

18. These biosafety guidelines have been developed on the basis of technical information presently available and may change in the future as more know-how becomes available. Therefore, revision of these guidelines will be a continuous process.

19. These biosafety guidelines will be supplemented with annexures explaining/elaborating concepts, procedures and protocols required in monitoring/implementation of the guidelines.

C H A P T E R 1

INTRODUCTION

"Biotechnology" is a collective term, encompassing a number of technologies, some old, others new. Plant and animal breeding are examples of traditional biotechnological methods. Making of foods, such as, bread, cheese, and yogurt; and medicines such as vaccines and hormones, are known products of old biotechnological methodologies. Modern biotechnology involves the more precise ability to effect genetic changes to produce improved or even new traits and includes such new techniques as recombinant DNA and gene cloning collectively referred to as "genetic engineering".

The precise definitions of "biotechnology" and of other terms such as "recombinant DNA" are of extreme importance when used as part of biosafety guidelines/regulations, particularly because they define the bounds of guidelines/regulatory jurisdiction. "Biotechnology" has been defined as processes using living organisms or parts thereof to make or modify products; and to improve plants, animals, or microorganisms for specific uses. "Recombinant DNA molecules" have been defined as molecules developed outside living cells by joining natural or synthetic DNA segments to DNA that can replicate in a living cell or DNA molecules that result from the replication of such DNA.

Modern Biotechnology has led to the development of new products/processes which manifest distinct advantages for the prevention and treatment of diseases, in the increased production of medically important compounds and improved vacancies. Recent breakthroughs include microbial production of human insulin, growth hormone, interferon, urokinase beta-endorphin, thymosin, alpha-3 somatostatin, tissue plasminogen activator. The list of biologically active compounds which can be made by Genetically Modified Organisms (GMOs) is increasing every day and it is believed that in the next 15-20 years, we will be able to produce all biological proteins by Genetically Modified Organisms. Through the use of powerful gene cloning and molecular immunological techniques, vaccines against malaria and hepatitis B have been prepared. Vaccines for trypanosomes, schistomyces, and leprosy are in the final stages of preparation. Monoclonal antibodies are being used for diagnostic and therapeutic purposes and this has made possible early diagnosis and cure of many dreadful diseases. Prenatal diagnosis has paved the way to bring down the incidence, of such genetic diseases as beta-thalassemia. Since heredity plays a part in virtually all diseases, including diabetes, cancer, cardiovascular disorders, multiple sclerosis, rheumatoid arthritis, schizophrenia, and a number of other disorders known to run in families, efforts are being made to sequence the causative genes to use this information in prenatal diagnosis in order to reduce the incidence, if not, completely eliminate these diseases.

In the industrial sector, modern biotechnology has paved the way for bacterial desulphurization of coal and petroleum; microbial production of fructose, polypropylene glycol, olefins and parafins; enrichment of precious metals, enhanced oil recovery, clearing of oil spills, sewage treatment and pollution control. The world production of organic chemicals is close to 100 million metric tons, about 90% of which are oxy-chemicals. It has been estimated that 60- 80% of oxy-chemicals and 20% of all other chemicals used by man, can be produced microbiologically.

Traditional plant breeding techniques are limited by sexual incompatibility barriers. In addition, valuable traits such as tolerance to specific herbicides and nitrogen fixation do not exist in plants. Biotechnological methods allow to overcome the genetic incompatibility barriers. Genes can be isolated from bacteria, viruses, fungi, plants or animals and made to express in easy and quick growing organisms. Genetically Modified Organisms (transgenic plants, animals and microorganisms) have been produced and used commercially. Globally over 70 different commercially important species of plants have been modified to incorporate mainly seven transgenic traits (Table-1). The important crops include maize, soybean, cotton, tomato, potato, alphalpha, petunia, rape/mustard, rice, wheat, beet, barley, chickpea (gram), cabbage and tobacco.

Table-1

Genetic transfer of traits in transgenic plants by recombinant DNA technology
• Herbicide tolerance • Insect resistance • Viral disease tolerance • Fungal disease tolerance • Product quality improvements • Male sterility traits • Others (production of metabolites/ chemicals, improvement of nutritional traits, incorporation of marker genes, stress resistance properties etc.)

1.1 REASONS FOR CONCERNS

As part of gene transfer technologies, target genes from unrelated organisms can be isolated and used after appropriate attachment of marker genes, the promoter and the terminator sequences for expression into new hosts to improve the productivity of organisms. Gene transfers are considered exotic because the genes from unrelated organisms are made to express in organisms which do not normally host them. Such mixing of genes from unrelated organisms might create natural imbalance by the transfer of genetic traits into organisms, the nature has so far not ordained. The consequence of flow of genes into unrelated organisms is not yet adequately known. There are apprehensions that certain transgenic organisms may be harmful or become harmful to economic plants, animals and human beings; and that manipulated genes or products thereof, if allowed to move around freely in nature, may pose potential hazards that include the following:

A. Changes in ecological roles:

Engineered changes in growth rate, reproductive output longevity, tolerance of physical and chemical factors (e.g., temperature, salinity, water legging, pesticides, etc.) can change the relative performance of GMOs with respect to naturally-occurring organisms. As a consequence, the performance of GMOs will be enhanced sufficiently to negatively impact other organisms. An often-cited example is the potential for increased weediness among herbicide-tolerant crops. Increased weediness could have negative impact on surrounding agricultural fields or on wild vegetation in nearby fields. Both impacts could have economic consequences, either directly, through loss of valuable crop-land, or indirectly, through loss of services in the ecosystem.

B. Changes in genetic relationships:

Many GMOs will retain the ability to interbreed with their non-engineered relatives. Interbreeding will allow the production of hybrid progeny expressing engineered traits. Such hybrids could be formed between GMOs and domesticated wild organisms. In either case, hybridization will alter the distribution of phenotypes within domesticated or wild populations, and will serve to change the role of the organism(s). In the most extreme cases, introgressive hybridization could lead to genetic contamination of economically important crops, or to extinction of native species or other species of local importance leading to loss of bio-diversity.

The potential for horizontal gene transfer among prokaryotes (e.g., microbes), from microbes to plants and vice-versa is of special concern. Horizontal gene transfer among eukaryotes, may occur by the action of transposons, but its occurrence is thought to be less frequent than in prokaryotes. Be as it may, such horizontal transfer of genetic material could allow engineered genes to move into populations other than the target host, with undesirable consequences.

C. Changes in allergenicity, toxicity, or nutritional composition of foods:

The presence of novel proteins in "familiar" foods could prove hazardous to individuals who suffer specific allergies to those proteins. Further, the production of toxins, even at very low levels, could have adverse effects on human health over the long term. In addition, foods grown for human consumption cannot be entirely isolated from other organisms and exchanges of genes in the environment. Thus, human food sources could become contaminated by novel genes introduced for purposes other than human consumption.

D. Indirect effects:

The indirect effects of releasing GMOs into the environment include changes in population mating structure, alteration of competitive hierarchies, and modification of the physical and chemical environments. Such alteration might lead to changes in population structure, and size through changes in species number. Indirect effects are difficult to predict, detect, and monitor, but may have substantial impact in the functioning of the ecosystem.

E. Ethical and social Issues:

The use of GMO's vis-a-vis religious belief, distribution of seeds to low-income farmers and product labeling, may give birth to ethical and social issues which can have serious consequences in the unique socioeconomic set-up in Pakistan. It is anticipated that the recently concluded International Treaties on Patent and Intellectual Property Rights such as WTO coupled with present level of investment by different Nations in R&D in biotechnology, will aggravate the situation by further widening the existing gap in the biotechnological capabilities of the developed and developing countries.

1.2 NEED FOR BIOSAFETY GUIDELINES/REGULATIONS

The concerns narrated above in Section 1.1 clearly show that the development and use of transgenic organisms in the open environment has to be looked at with caution to ensure safetyof user and the environment. This has raised the need to develop and adopt safety protocols during laboratory experimentation as well as during eventual use of Genetically Modified Organisms (GMOs) and the products thereof. As a rule, research on GMOs is carried out by competent researchers who are fully conscious of good laboratory practices and the acceptable safety of releasing the GMOs into the environment. Nevertheless, progress and accomplishment made during the past decade has, evoked concerns among researchers themselves. The main concern is that human desire to rapidly progress coupled with pressures from research financers to rapidly produce, can result in complete forgetfulness of the appropriate need to adopt good laboratory practice and/or necessary experimentation/field trials that must be done in order to be able to eventually develop and release transgenic and/or products thereof, into the open environment. It is therefore necessary to develop appropriate biosafety procedures to guide scientific researchers during their laboratory work to develop Genetically Modified Organisms and to enable the eventual use of Genetically Modified Organisms as well as products thereof in the open environment for economic gains. The main goal of developing biosafety procedures is to discern, as far as possible, the potential for harm to the environment and/or human well-being stemming from GMO's and the products thereof.

A number of developed countries have prepared biosafety guidelines for both laboratory research and field applications of GMO's and their products. Such guidelines differ from one country to another, however, the guiding principle is to ensure safety and minimize all the risks which are likely to occur, encountered or subsequently generated. It took the United States and Europe nearly two decades to develop regulations and other oversight mechanisms pertaining to biotechnological work. There is no easily stated unifying principle and as a consequence, many of the guidelines developed by different regulatory agencies/countries at different times have not been consistent in their approaches and the differences are attributed mainly to different legislative mandates. It seems doubtful that there ever will be a single or unified international basis of "biotechnology regulation". The subject area actually involves a complex variety of technologies and products thereof. Further, individual viewpoints, guided by religious and social believes, on each of these diverse subjects can differ dramatically. This diversity of viewpoint can create, and has resulted in, different biosafety regulations.

Many developing countries have used guidelines of developed countries as references and then modified or amended in compliance with the relevant laws, regulations, and local conditions. At the international level, UNIDO, FAO, WHO, UNEP, and other such agencies have prepared

guidelines which have helped developing countries to formulate their own biosafety guidelines. As far as Pakistan is concerned, there is an immediate need to formulate national biosafety policy to regulate all biotechnological work in the country in order to eliminate the concerns narrated in the previous pages. Such a policy will, undoubtedly, promote R&D efforts in biotechnology in the country and also help to foster collaborative ventures between local and foreign scientists/laboratories interested in joint experimentation, laboratory testing or field trials of GMOs in Pakistan. This is the main rationale upon which the following Biosafety Guidelines are developed.

The objective of these guidelines, prepared by the National Biosafety Committee (NBC), is not to enforce stringent regulations such that they will impair activities in the research and development of recombinant DNA technology in the country. At the same time, the objective is also not to be too lenient to allow unintentional negligence leading to misuse and irresponsibility by certain researchers or laboratories. The scope of these guidelines embraces all works related to gene manipulation employing recombinant DNA technology for all purposes including the development of transgenic plants, animals and microorganisms, production of vaccines, industrial manufacturing of Genetically Modified Organisms and products thereof, and their releases into the environment for field trials and for commercial purposes. The National Biosafety Committee realizes that these guidelines are far from complete and future amendments and revisions are unavoidable. As more information becomes available, it will be incorporated to make the present guidelines more feasible and supportive of the overall development of biotechnology in the country.

1.3. BASIS OF BIOSAFETY GUIDELINES

The biosafety guidelines described on the succeeding pages, have been developed to prevent possible adverse effects resulting from recombinant DNA laboratory work and the deliberate release of resultant Genetically Modified Organisms and products thereof, on human health and environment. Considering the purposes of these guidelines, regulated material includes all genetically modified materials (DNA and RNA preparations, viroids, viruses, cells and organisms, modified or constructed through genetic engineering), derivatives thereof and the wastes or by-products of genetic engineering practices (containing viable organism or otherwise). The Guidelines consist of two parts; the first part relates to regulated work in laboratories and fields. The second part deals with procedures for approvals which must be obtained to deregulate the regulated materials to allow their free movement and commercial uses.

1.3.1. All regulated works are classified according to level of anticipated risk and safety, into three categories:

A) Work bearing minimal risk,
B) Work bearing low risk,
C) Work bearing considerable level of risk.

It is important to divide all works into various categories according to anticipated levelsof risk so that risk management and control measures can be taken accordingly (details are in appendices).

1.3.2. The proposed guidelines suggest three tiers for monitoring and implementation.

Firstly, the Principal Investigator and researchers are responsible to themselves and to the community. The monitoring and inspection will be done by the Institutional Biosafety Committee (IBC). In the whole set-up, the IBC is an important organ and occupies a pivotal position. It forms the foundation of the entire set-up. The Committee will receive applications, propose measures for laboratory set-up as well as planned release and effectively monitor them. All information/data that needs to be submitted to the subsequent two tiers, will have to go through the IBC which therefore, must consist of people adequately qualified to understand the associated risks and evaluate them accordingly.

Secondly, a Technical Advisory Committee (TAC) which will have the major responsibility to technically review all applications for licensing and ensure that the GMOs or any product under consideration has gone through proper risk assessment under these guidelines.

Thirdly, a National Biosafety Committee (NBC), set-up under the Ministry of Climate Change & Environmental Coordination, will act as the executive body for the overall monitoring, risk management and commercial release of all regulated materials. Chapter-5 gives details on the composition and functions of the various committees.

1.4. SCOPE OF GUIDELINES

1.4.1. Laboratory Work

These guidelines are pertinent to all research whether conducted in laboratories of teaching and research, research and development institutes or private companies-involved in the uses and applications of GMO's and products thereof.

The question whether a particular endeavor rests within the scope of these guidelines, should be addressed by preparing a proposal according to the format in Chapter-10, and submitting it to the Institutional Biosafety Committee (IBC). In the event, an IBC has not been properly established, the same proposal should be submitted to the TAC through the controlling ministry for consideration. Researchers, with even the slightest doubt in these matters, are encouraged to seek guidance from their parent administrative Ministry or the Ministry of Climate Change & Environmental Coordination, Islamabad.

1.4.2. Field Work

These biosafety guidelines cover all work involved in the field trial of genetically manipulated plants, animals and microorganisms. As a rule, laboratory work must precede all field trials. As a standard practice, genetically manipulated organisms from laboratory work must be field tested before commercial release into the environment. The proposed field trials should:

i) repeat the experiments made during laboratory work, and verify the results from tests performed at the laboratory level.
ii) gather precise and accurate information/data on the stability, expression, and hereditary transmission of transgenes under field conditions.
iii) assess the viability (e.g. survival, propagation, competitive ability) of genetically manipulated organisms under field conditions.

iv) assess the adaptive or evolutionary potential of genetically manipulated organisms under changing environmental conditions.

& v) asses the overall environmental impact.

1.4.3. Commercial Release of Regulated Materials

These guidelines also cover procedures needed to deregulate regulated materials for commercial purposes including import and commercialization of GMOs intended for direct use as Food or Feed or for Processing. As a standard practice, sufficient information should be available from the field trials to make a decision whether the GMO or its product can be released for public use. In this regard, it is extremely important to include all information/considerations as well as data which formed the basis of deregulation/commercial release in another country. The procedures for preparing applications and assessment by different regulatory bodies is presented in Chapter-12 and 13 accordingly.

C H A P T E R 2

GUIDELINES FOR LABORATORY WORK

The guidelines classify all laboratory work into three main categories.

2.1. RISK CATEGORY 1 WORK:

2.1.1. Biosafety:

Risk Category 1 work appears to convey minimal risk, nevertheless, such work must be undertaken in compliance with standard practices for conventional microbiological laboratories. Experiments involving pathogenic organisms should conform to suitable containment and precautionary measures including personnel training and instructions. Laboratory staff should be familiar with all pathogenic organisms under study and aware of the appropriate safety procedures required (Appendix-1).

2.1.2. Liaison with the Institutional Biosafety Committee (IBC)

All work to be considered minimal risk status must initially be referred to the IBC for notification and approval. In the absence of an IBC for the institution supporting the work, the responsible workers should refer directly to the concerned ministry for endorsement of their proposals. A standard form, to be submitted to the IBC for consideration of project exemption, is outlined in Chapter-13. Individual IBCs are at liberty to design additional forms as well, following the terms of the standard proposal for request of exempt status. The Institutional Biosafety Committee will make the final decision as to whether a proposal warrants Risk Category 1 or other categories in the light of available information. As such, no work which falls under the scope of these guidelines should be initiated unless approval is awarded by the IBC, regardless of the risks.

For the work that has already been considered as risk category 1, further modifications of consequence, to any component of the experimental system, which may place this status in jeopardy, must be referred to the IBC for its approval, a second time. A revised proposal together with the original proposal should be submitted to the institutional biosafety committee which will review the amendments and judge whether such amendments place the work in higher-risk category. The concerned ministry will be informed if the IBC has placed the amended work in risk category 2 or 3.

2.1.3. Experiments characterized as Risk Category 1

Experiments characterized as risk Category 1 include:

A. Tests involving organisms that naturally exchange genetic material, provided that the donor and the recipient are of the same species or that the donor species can exchange genetic material with the recipient species under natural circumstances. A list of such combinations is found in Appendix-2. and in which the donor DNA exhibits all the following properties:

- is not derived from microorganisms which cause diseases in humans,plants, or animals.
- represents or comprises no more than 2/3 of any complete viral genome and is employed in such a manner as to disallow/prevent the possible regeneration of live viruses (as opposed to such work wherein the hosts carry the missing segments of viral genomes or whereby regeneration is made possible under the context of ensuring propagation sequences).
- does not code for proteins which regulate the growth of mammalian cells (e.g. product of oncogenes), for cytotoxic proteins, or for toxins, to vertebrates, with an LD50 of less than 100ug/kg (Appendix-3).

B. Fusion of protoplasts among non-pathogenic microorganisms or between plant cells (embryo rescue).

C. Fusion of cells derived from 'higher' animals, but which does not result in a viable organism such as in the creation of hybridomas without the use of a viral stimulator (e.g. EBV for production of monoclonal antibodies.

2.2. RISK CATEGORY 2 WORK

2.2.1. Biosafety

Risk Category 2 work may pose "low" levels of risks towards laboratory personnel, the community or the environment and necessitates, at the very least, containment level C1. Situations involving whole plants and animals should normally be conducted under PH1 plant glass houses or C1A animal houses. Certain types of work (IBC will decide on the basis of expert opinion on the subject) however, may call for further safety precautions or high levels of physical containment (e.g. special containment conditions in some animal houses designed for transgenic species) because the DNA concerned, or segments and fragments thereof, may conceal hazards or cause disease. Such work will be negotiated on a case-by-case basis with the IBC. Regulations and criteria for the procedures, facility design and containment features at the various biosafety levels are outlined in Appendices 4 through 11.

2.2.2. Liaison with the Institutional Biosafety Committee

The project supervisor or head researcher has the primary responsibility for identifying the nature of potential hazards within the laboratory and for determining additional precautions -- supplementing the relevant standards laid down in Appendices 4 through 11 -- appropriate to the level of risks and concerns in this category of work. The need for additional procedures and conditions are to be in line with and address the specific risks. The project proposal from the project supervisor must be referred to the IBC for consideration. The IBC shall evaluate the proposed ambient working conditions and containment resources and determine the sufficiencyof these provisions. Considering the risk assessment results, the IBC may also impose special requirements on the work at hand. The work will start only after obtaining IBC approval. The IBC must then forward the proposal and its assessment thereof, to the NBC via TAC record- keeping.

2.2.3. Experiments Characterized as Risk Category 2 Include the Following:

A. Work with previously approved host/vector systems (Appendix-2) but in which the genetic material inserted exhibits one or more of the following properties.

- codes for proteins regulating cellular metabolism, growth, or division.
- represents a pathogenic determinant.
- represents an uncharacterized DNA or RNA sequence derived from microorganisms which cause diseases in humans, plants, or animals.

B. Work with non-approved host/vector systems.

C. Genetic engineering of modified whole plants. Supplementary information form required for submission is outlined in Chapter-10.

D. Altering the genome of oocytes, zygotes, or early embryos, through any means, to the extent that a novel organism results.

- Genetic manipulation work on live animals (microorganisms notwithstanding) to the extent that a novel organism results.

2.3. RISK CATEGORY 3 WORK

2.3.1. Biosafety:

Risk Category 3 distinction is placed on work which may pose a substantial level of risk to laboratory personnel, the community, or the environment. Gene-therapy work for which the character and degree of the risks are as yet uncertain fall under this category. With such an array under the umbrella of Risk Category 3, appropriate containment levels are far more rigid and may vary considerably depending on the inherent nature of the experiment and on risk assessment results, containment level C1 may be adequate for some types of work whereas other situations may demand higher levels of containment and experienced personnel.

2.3.2. Liaison with the Institutional Biosafety Committee

The IBC serves, in part, as a conduit for the flow of information between the researchers and the NBC forwarding proposals, assessments and recommendations. Work is prohibited before consent which is granted by the IBC/NBC. Only after both biosafety committees have inspected and reviewed the project proposals, and advised the relevant authorities on any measures that research work should be allowed.

2.3.3. Work Characterized as Risk Category 3 Include the Following:

A. Application of genes determining pathogenicity in microorganisms other than the approved host microorganisms listed in Appendix-2.

B. Transfer of whole viral genomes, viroids or genetic fragments known to initiate infection in humans, plants, or animals. In general, work using gene sequences less than 2/3 of any complete viral genome and work involving genetic material lacking components vital to the proper functioning of problem operons, to replication or to the packaging of new viral particle are excluded from this subcategory, provided that experimental conditions disallow/prevent the regeneration of live, infectious viruses.

C. Recombination between complete viral genomes, viroids and/or complementary fragments thereof determined to be of infectious or pathogenic nature.

D. Alteration of host range for infection, virulence, or infectivity.

E. Work using viral vectors capable of infecting human cells.

F. Work using microbial hosts or vectors which are human, plant or animal pathogens with the exception of those listed as approved hosts and vectors under Appendix-2.

G. Application of genetically manipulated DNA sequences coding for proteins known to regulate cell growth or to be toxic to human cells.

H. Work involving toxin producers including:

- DNA coding for toxins with an LD50 of less than 100ug/kg (Appendix-3) or otherwise, exhibiting high levels of gene expression regardless of how low the toxicity of the protein encoded.
- Uncharacterized DNA from toxin-producing microorganisms which may contain unfamiliar toxin-determining sequences.

I. Use of defective vector/helper virus combinations which heightens the tendency of regenerating non-defective recombinant viruses.

J. Genetic engineering of animals to secrete or to produce viruses, through the injection of viral genetic material or of whole viral genomes into embryos.

K. Propagation through cloning.

- Gene-therapy work through genetic modification of any nature.

2.4. UNCHARACTERIZED WORK

2.4.1. Works which do not fall under Categories 1,2 and 3 because it deals with completely uncharacterized materials, but which nonetheless fall under the coverage of these Guidelines as defined, shall be treated as Risk Category 3 work. Considering this special provision, researchers attempting such uncharacterized work must adhere to the requirements imposed by Risk Category 3 distinction.

2.4.2. The standing of any research work is liable and subject to change in either direction (from lower to higher risk categories or vice versa) as appropriate new information or further procedural revisions are implemented, subsequent to relevant notification. Project supervisors must submit revised project proposals to the IBC for consideration and recommendation before adopting radical operating procedures or substantially changing any parameter of the work (especially approaches to physical and biological containment) which may introduce novel risks, delimit new biosafety levels, or warrant change of classification.

2.4.3. Researchers who wish to restrict access to information of commercial significance (e.g. trade secrets or confidential business reports) provided to the various committees, should mark the relevant sections "Commercial-in-Confidence".

C H A P T E R 3

GUIDELINES FOR FIELD WORK

3.1. GENETICALLY MODIFIED MICROORGANISMS

Field work with genetically modified microorganisms must first take into consideration the nature or character of the biological system, as follows:

3.1.1. For microorganisms, considered to have a history of safe use in the field, work may proceed in accordance with the basic standards appropriate to the particular microorganism. Those microorganisms,

A. which are from a strain that has been involved in previous documented field work;
B. which perform the same functions as strains that have been involved in previous documented field work;
C. which remain confined to sites and surroundings that resemble previous field conditions, are considered to have a history of safe use.

3.1.2. For experimental microorganisms which do not meet the above conditions, the work may proceed under appropriate containment levels as presented in Regulations and Containment Section. The proposed measures of containment must observe any one or more of the following conditions:

A. There is appropriate biological containment, where:

- Microorganisms are rendered non-reproducible before being field tested; or
- Modifications are done to limit the survival of microorganisms outside, and to confine microorganisms within target areas.

B. Genetic inserts and constructs may be exchanged or transferred to other microorganisms only in a restricted area.
C. There are physical arrangements to contain the dispersal of microorganisms within the target areas or site of trial.

3.1.3. In the case of those microorganisms which do not have a history of safe use in field, the work may proceed with a preliminary risk assessment to determine the full range of possible environmental effects. Those microorganisms recognized as "problematic" that have been engineered for the,

A. Sustenance and nourishment of plant species, which may therefore provide nutrients in excess to disrupt the chemistry of associated plants;
B. destruction of toxic residues which may prompt secondary ill-effects;
C. biological control of plant pests which may overwhelm target species and thus produce toxic or pathogenic metabolites, spreading ill-health in wild populations at the trial site.

3.2. GENETICALLY MODIFIED PLANTS

Field work with genetically modified plants must first take into consideration the nature or character of the biological systems, as follows:

3.2.1. For experimental plants considered to have a history of safe use in the field, the work may proceed in accordance with the basic standards appropriate to the particular plant. Those plants are considered to have safe history that have been modified by,

A. conventional breeding practices (e.g. selective breeding, mutagenesis, protoplast fusion or embryo rescue) and/or having inherent characteristics typical of modified plants from conventional breeding practices,
B. the introduction of genetic inserts that are known to be harmless and non- hazardous to the environment.

3.2.2. For experimental plants which do not meet the conditions of 3.2.1, work may proceed under appropriate containment level and criteria, as presented in Chapter-4. The said measures of containment must observe any one or more of the following conditions:

A. There is no cross-hybridization.
B. There are arrangements to contain the dispersal of plants and plant materials.
C. Introduced gene expression is stable, and does not fluctuate with changing environmental conditions.

3.2.3. In the case of those plants which do not have a history of safe use in the field, work may proceed with a preliminary risk assessment to determine:

A. Effects on the ecology of the trial site.

- Increased resistance to diseases and pests.
- Propensity for weediness.
- Effects on other target and non-target organisms.

B. Effects on the ecology in the open environment

- Potential for cross-hybridization.
- Promotion of and stimulus for the growth and development of weeds.
- Invasion of wild populations beyond the trial site.

C. Effects on other elements in the surroundings.

3.3. GENETICALLY MODIFIED ANIMALS

Breeding of genetically modified animals should be conducted in accordance with the following:

A. Genetically modified animals should be bred in breeding containers clearly distinguishable from other containers used for breeding non-engineered animals. Further, genetically engineered animals be individually distinguished and housed, however, if individual distinction becomes difficult, small laboratory animals may be managed according to breeding groups.
B. Wastes relating to genetically modified animals (including dead bodies should be disposed off after sterilization and incineration as necessary.

C. In transporting genetically modified animals outside the work area, containers possessing sufficient strength and structure to prevent escape, should be used.

D. The word "Handle with Care" in "distinguishable ink" should be clearly displayed on all containers that house genetically modified animals.

E. Maintenance and management facilities and equipment and so on used for these studies should be performance – tested at the time of installation and periodically and thereafter to maintain original performance level.

F. A sign stating genetically modified animals should be posted at each work area.

G. The work area should be kept clean.

H. Working clothes should be worn in the work area only.

I. When transferring genetically modified animals to other facilities and other personnel, the person responsible must notify receiving personnel of all the relevant information.

C H A P T E R 4

REGULATIONS AND CONTAINMENT DURING FIELD TESTS

4.1. GENETICALLY MODIFIED MICROORGANISMS

4.1.1. Experimental Microorganisms with a History of Prior Work

Field testing of microorganisms with a history of prior field work, still requires submission of a project proposal to the IBC which shall evaluate the sufficiency of biosafety provisions. Measures for the control and containment of field work should observe relevant, regulations and must address the particular microorganism(s) under study. The work will begin, only after receiving IBC endorsement. The IBC must forward all proposals and assessments, to the NBC through the TAC for records and information.

4.1.2. Experimental Microorganisms with No History of Prior Field Work

Field testing of experimental microorganisms with no history of prior field work should proceed under the advice, counsel and direction of the IBC and NBC. In such cases, approvals will be based on the biosafety concerns that may be gathered from the written proposals submitted. The project supervisor is prohibited from initiating work before consent is granted bythe NBC.

Considering the risks involved with untested experimental microorganisms, measures for the control and containment of field trial must set aside provisions for the following:

A. The medium for testing experimental microorganisms (e. g. soil, water, or air) is regulated and contained, at levels approved by the NBC.
B. The boundaries of testing areas need to be clearly demarcated, and posted with "No Entry" signs. Use of testing areas is strictly regulated.
C. The dispersal of experimental microorganisms is monitored closely with a reliable and effective technique, approved by the NBC.
D. Arrangements are made to destroy/inactivate experimental microorganisms, at the conclusion of work.
E. Other measures which the NBC or IBC deem suitable.

4.2. GENETICALLY MODIFIED PLANTS

4.2.1. Experimental Plants with a history of Prior Work

Field testing of experimental plants with a history of prior field work at home or aboard, still requires submission of a proposal to the IBC which shall evaluate the sufficiency of biosafety provisions. Measures for the control and containment of field work should observe relevant regulations. In these situations, work may begin only after receiving IBC endorsement. The IBC must forward all proposals and the committees assessments, to the National Biosafety Committee through concerned ministry for records and information.

4.2.2. Experimental Plants with No History of Prior Field Work

Field testing of experimental plants with no history of prior field work should proceed under the advice, counsel and direction of IBC, and the concerned ministry. In such cases, approvals will be based on the biosafety concerns that may be gathered from the written proposals submitted. The project supervisor is prohibited from initiating work before consent is granted by the NBC.

Considering the risks involved with untested experimental plants, measures for the control and containment of field trial must set aside provisions for the following:

A. Contained tests may be conducted in plant glass houses, on site. The scale and period of contained cultivation is appropriate to both the nature of the investigation, and the nature of the particular plant.
B. The site chosen is suitable to the particular plant under study. Test plots are fenced in and isolated from feral populations. "No Entry" signs are put up at regular intervals around the perimeter.
C. Arrangements are made to collect, burn and destroy experimental plants and plant materials at the conclusion of work.
D. The cultivation of plants is surveyed and directed by the IBC, at regular intervals, as appropriate to the growth or developmental patterns of the particular plant.
E. Other interests, which the NBC or IBC deems suitable.

4.3. GENETICALLY MODIFIED ANIMALS

A. Field trials of genetically modified animals with history of prior field work at home or abroad, may begin after receiving IBC endorsement. The IBC must forward all proposals and the committee's assessment to the National Biosafety Committee (NBC) through the concerned ministry for records and information.

B. Field testing of experimental animals with no history of prior field work should proceed under the advice, council and direction of IBC and the concerned ministry. The project supervisor is prohibited from initiating work before consentis granted by the NBC. Considering the risks that may be associated with the uses of genetically modified experimental animals, all measures for the control and containment of field trials must be observed.

C. Please also see pages 31-32 & 34.

C H A P T E R 5

COMPOSITION AND FUNCTIONS OF VARIOUS BIOSAFETY COMMITTEES

The implementation of bio-safety practices in genetic engineering and biotechnological work will be supervised by the National Biosafety Committee (NBC), the Institutional Biosafety Committee (IBC) and the Project Supervisor.

The various authorities retain different powers and responsibilities yet are bound by the common objectives of enforcing and of preserving the integrity and the intent of national guidelines.

All three committees will have the powers to ensure adherence to the specifications of national guidelines for the safety of personnel, the community and the environment from the risks that may be associated with the study. In case of non-compliance of prescribed biosafety measures, whether deliberate or unintentional, the Committee which accorded approval, will have the powers to stop work after giving a show cause notice and proper investigation through the relevant IBC. The NBC will have the powers to prescribe additional safety measures/conditions to intervene in any other manner through the relevant IBC.

In the discharge of its functions and responsibilities, the various committees may co-opt additional members, form sub-committees and adopt other such measures which may facilitate its work.

5.1. THE NATIONAL BIOSAFETY COMMITTEE (NBC)

Constituted by the Ministry of Climate Change & Environmental Coordination

5.1.1. Composition of NBC

(a)	Secretary, Ministry of Climate Change	Chairman
(b)	Chairman, Pakistan Agriculture Research Council (PARC)	Member
(c)	Member (Science), Pakistan Atomic Energy Commission	Member
(d)	Representative, Government of Punjab	Member
(e)	Representative, Government of Balochistan	Member
(f)	Representative, Government of Sindh	Member
(g)	Representative, Government of KPK	Member
(h)	Representative, Environmental Protection Agency, Azad Jammu & Kashmir	Member
(i)	Representative, Gilgit Baltistan Secretariat, Forest Wildlife and Environment Department	Member
(j)	Representative, Ministry of National Food Security & Research	Member
(k)	Representative, Ministry of National Health Services, Regulation and Coordination	Member
(l)	Representative, Higher Education Commission	Member
(m)	Representative, Ministry of Science and Technology	Member
(n)	Representative, Ministry of Commerce & Textile Industry	Member
(o)	Representative, Federal Board of Revenue	Member
(p)	Representative, Ministry of Industries & Production	Member

(q)	Director General, Pakistan Environmental Protection Agency	Member/ Secretary
(r)	Director General, Department of Plant Protection, Karachi.	Member
(s)	Chairpersons of concerned Institutional Biosafety Committees.	Members

5.1.2. Functions of National Biosafety Committee.— **(**1) The National Biosafety Committee shall perform the following functions; namely:-

(a) to establish standards and procedures for risk assessment and labeling of living modified organisms, substances or cells and products thereof.

(b) to consider application(s) for import, export or commercial release of living modified organisms, and on the recommendations of Technical Advisory Committee allow release or reject applications after reviewing the risk assessment carried out in accordance with the biosafety guidelines, the procedures established under clause (a) and any other reliable information.

(c) to ban or restrict import, export, sale, purchase or trading of any living modified organism causing or likely to cause risk to public health, safety or environment.

(d) to develop linkages with foreign biosafety committees and relevant agencies to ensure that genetic manipulation practices in Pakistan address international biosafety concerns and observe universal codes of conduct.

(e) to cooperate with other relevant federal or provincial authorities overseeing the import and release of living organisms and formulate guidelines for the identification, inspection and regulation of transgenic species exotic organisms and others.

(f) to restrain on the advice of Technical Advisory Committee any person, authority or institute involved with genetic manipulation experiments of potential hazards.

(g) to facilitate exchange of technical expertise to various research institutions and regulatory agencies in setting up appropriate experimental conditions.

(h) to facilitate all levels of supervision of genetic manipulation work by assisting other regulatory bodies including Institutional Biosafety Committees, in establishing pertinent codes disciplines and guidelines for the appraisal of biohazards and the management of bio- safeguards.

(i) to coordinate efforts of Institutional Biosafety Committees and inform and educate the public on biosafety issues and on proposed national policies.

(j) to ensure that laboratory, field work and commercial release of Genetically Modified Organisms and their products conforms to the National Biosafety Guidelines.

(k) to prepare and provide to Institutional Biosafety Committees the various notifications and assessment forms, biosafety guidelines, related documents and assorted signs for facilities.

(l) to inform the various institutions engaged in genetic manipulation work about new developments in biosafety so as to avoid exposure of laboratory personnel, the community or the environment to undue risks.

(m) to coordinate efforts between pertinent government agencies and private organizations to maintain safety levels in biotechnological work and to prepare them for biological emergencies.

(n) to certify high-level laboratories, plant glass houses and animal houses intended for use in high-risk work. Upon request by the institution, and at the earliest convenience,the National Biosafety Committee may inspect a facility and either issue certification,or recommend additional precautions, if elements of the facility are determined to be inadequate to support the types of risk or hazard companying work requiring such physical containment.

(o) to inspect high-level laboratories and containment facilities on a regular basis. The National Biosafety Committee may inspect laboratories and facilities of containment level C2, PH2. and C2A, as specified in the bio safety guidelines, equivalent or higher at any time subsequent to certification without prior notice.

(p) to inspect systems equipment and instruments governing ambient biosafety levels in genetic manipulation laboratories.

(q) to keep information of commercial significance confidential from public domain if so, requested in writing by applicant, person or institution or organization.

(r) to monitor the safety related aspects of on going research projects and achievements involving genetically engineered organisms/hazardous substances or cells and products thereof.

5.1.3. Postal Address of NBC

C/o Director General (Pak-EPA) Tel: 92-51-9250713
(Ministry of Climate Change & Environmental Coordination) Fax: 92-51-9206343
Pakistan Environmental Protection Agency
Plot 42, Street 6, H8/2, Islamabad, Pakistan E-mail: dg@environment.gov.pk

5.2. TECHNICAL ADVISORY COMMITTEE (TAC)

5.2.1. Composition of TAC

Technical Advisory Committee:—(1) The Federal Government shall, by notification in the official Gazette, establish a Technical Advisory Committee consisting of the following members, namely:-

i)	Director General, Pakistan Environmental Protection Agency	Chairperson
ii)	Director, National Institute of Bio Technology and Genetic	Vice

	Engineering, Faisalabad	Chairman
iii)	Executive Director, Pakistan Medical Research Council (PMRC), Islamabad	Member
iv)	Member (Science), Pakistan Council of Industrial and Scientific Research (PCSIR), Islamabad	Member
v)	Executive Director, National Institute of Health (NIH), Islamabad	Member
vi)	Director, Centre for Molecular Genetics, University of Karachi	Member
vii)	Representative, Centre for Applied and Molecular Biology, Lahore	Member
viii)	Relevant Technical Expert (Plant Sciences), Pakistan Agriculture Research Council (PARC), Islamabad	Member
ix)	Relevant Technical Expert (Animal Sciences), Pakistan Agriculture Research Council (PARC), Islamabad	Member
x)	Technical Expert, Environmental Protection Agency Punjab	Member
xi)	Technical Expert, Environmental Protection Agency Sindh	Member
xii)	Technical Expert, Environmental Protection Agency Khyber Pakhtunkhwa	Member
xiii)	Technical Expert Environment, Sports & Youth Affairs Department, Balochistan	Member
xiv)	Technical Expert, Environmental Protection Agency Azad Jammu & Kashmir	Member
xv)	Technical Expert, Environmental Protection Agency Gilgit Baltistan	Member
xvi)	Representative, Intellectual Property Organization (IPO)	Member
xvii)	Representative, IUCN – The World Conservation Union Pakistan	Member
xviii)	Expert, Sustainable Development Policy Institute (SDPI), Islamabad	Member
xix)	Expert Action Aid Pakistan	Member
xx)	Representative, Ministry of Textile Industries, Islamabad Co-opt Member	Member
xxi)	Representative, Federal Seed Certification & Registration Department (FSC&RD), Islamabad Co-opt Member	Member
xxii)	Director, Pakistan Environmental Protection Agency	Member / Secretary

The Director, Pakistan Environmental Protection Agency shall act as Secretary of the Technical Advisory Committee.

The Committee may co-opt any technical representative from any province.

5.2.2. Functions of Technical Advisory Committee: The following shall be the functions of the Technical Advisory Committee, namely:-

(a) to examine applications and recommend National Biosafety Committee on permitting license? or otherwise laboratory work, field work or release of living modifiedorganism, substances, cells, and products thereof;

(b) to review and control of safety measures adopted while handling large scale use of genetically engineered organisms/classified organisms in research, developmental and industrial production activities;

(c) to review research methodologies in genetic engineering and recombinant DNA work at the international level and assess the associated risks to guide relevant institutions;

(d) to monitor release of engineered organisms or products into environment and to oversee field applications and experimental field trials;

(e) to provide information or data inputs to National Biosafety Committee upon surveillance of approved projects under industrial production, and in case of environmental releases with respect to safety risks and accidents; and

(f) to supervise directly or through any person authorized on this behalf/in this regard? the implementation of the terms and conditions laid down in connection with theapprovals accorded by the National Biosafety Committee.

5.3. THE INSTITUTIONAL BIOSAFETY COMMITTEE (IBC)

Anyone engaged, or with the intent to engage, in the purchase, construction, propagation or field release of Genetically Modified Organisms or components must each arrange for the establishment of an Institutional Biosafety Committee (IBC) to serve as the administrative authority on matters of biosafety and on compliance with these Guidelines. To grant the IBC freedom to exercise the full extent of its powers in undertaking all its functions and responsibilities, the pertinent institutions and organizations must appoint appropriate and capable individuals to the IBC and support the needs and demands of the committee. In addition to the IBC, institutions, and organizations, particularly those engaged in industrial-grade or other large-scale work, are encouraged to recruit a Bio-Safety Officer (BSO) to work in conjunction with various biosafety committees.

Anyone dealing with GMOs or LMOs, which may encounter difficulties in constituting an IBC, may alternatively request other IBCs or TAC to bear the responsibility for monitoring and supervisingthe biosafety aspects of their work. Agreements of this nature must be formulated between the parties involved and the NBC must be notified of the proceedings. A representative of the smaller institution requesting assistance must maintain close ties with the non-affiliated IBC or more desirably, serve as an acting or even as an honorary member of the committee.

5.3.1. NBC Certification

For the IBC to receive formal endorsement from the NBC, the pertinent institution must submit to the NBC (through TAC) for information and record, a completed notification form (see appendix), detailing the academic and professional history, faculty and qualifications of each member appointed to the committee and the following information:

- IBC membership.
- designated Bio-Safety Officer, where applicable.
- a list of current projects (indicate risk assessment category).
- a list of laboratories approved for recombinant DNA work (indicate category of containment).
- a list of the institution's plant glass houses and animal houses, certified and intended for work with transgenic species (indicate category of containment).

5.3.2. IBC Composition

The head of the institution will appoint this committee. With hindsight, these Guidelines are meant, above all, to simply help and provide a framework for institutions, engaged in genetic manipulation work, to consider issues of risk assessment and Biosafety. In as much as the intent of these Guidelines should always be respected, the primary responsibility for maintaining various standards and ensuring biosafety rest with the institutions and the researchers concerned, and should never be wholly dependent upon national guidelines or upon the NBC. The IBC, in particular, represents the most important element in the domain of biosafety--whether the specifics involve supervising genetic manipulation work, attending to the health of personnel, etc.--and therefore should comprise members of high caliber and considerable experience to assume the functions and responsibilities. In addition, the Chairperson of the IBC should retain a senior position under the pertinent institution possibly the Director himself/herself to ensure swift adoption of the committee recommendations.

To supervise laboratory genetic manipulation work, the IBC shall comprise no less than three members, with the following suggested distinctions:

- An individual with the abilities to evaluate, assess and advise genetic- manipulation work for the institution. Ideally, such an individual should be a respected authority in the particular field of research supported by the institution (e.g. plant genetics, human physiology, virus life-history, etc.).
- An engineer with the necessary expertise and practice to examine and assay the integrity of facilities instruments and tools governing ambient biosafety conditions (optional).
- A Bio-Safety Officer, where applicable.
- A subject expert from the Institution.
- A representative/member of TAC or NBC or a public representative.

Recognizing that biosafety issues evoke many disciplines, the IBC should also consider the prospect of establishing working arrangements with individuals--knowledgeable in relevant areas.

The institution should appreciate the critical role assumed by the supervising IBC and should thus grant principal authority on biosafety concerns to the committee enabling it to exercise its powers in undertaking all its responsibilities and offer criticism and advice without contest.

5.3.3. Institutional Biosafety Committees.— The head of the institution/organization related to biotechnology shall notify Institutional Biosafety Committee with the following minimumcomposition:

(a) Head of the institution	Chairperson
(b) Subject expert (s)	Members
(c) Social Scientist/economist (for social impact)	Member
(d) Representative of civil society	Member

5.3.4. Functions of Institutional Biosafety Committee.— The Committee shall perform the following functions, namely:-

(a) to assist in the activities of National Biosafety Committee and Technical Advisory Committee;

(b) to assist researchers in undertaking risk assessment, organizing training programmes and harmonizing experimental conditions with biosafety guidelines;

(c) to determine additional safeguards and draft supplementary operating instructions for work at the institution, in line with and addressing the specific risks and concerns uncovered;

(d) to evaluate the qualifications of researchers involved in biotechnological projects and assess whether each retains a thorough understanding of good microbiological practices necessary for the supervision of students, assistants and junior personnel;

(e) to monitor all regulated work under progress within the institution and counsel the proponents on issues of biosafety and on compliance with biosafety guidelines on a regular basis, or as requested;

(f) to serve where appropriate, as a gateway for the flow of information, ideas and opinions between the National Biosafety Committee and the research teams;

(g) to maintain and update a directory of all personnel engaged in activities at every biosafety level and to instruct new personnel on the correct laboratory or field practices, emergency procedures and equipment operation at the relevant level;

(h) to ensure health of laboratory and field personnel as may deem necessary from medical records;

(i) to liaise with National Biosafety Committee and Technical Advisory Committee on import, export, manufacture, process, use or sale of any Genetically Modified Organisms/substances or cells and products thereof for the purpose of research;

(j) to withhold funds and or use administrative authority to immediately refrain programmes if biosafety guidelines are violated;

(k) to prepare and implement the institutional emergency and response plan according to the details provided in the manuals and guidelines prepared by National Biosafety Committee.

(l) to assess all projects referred to it, and on the basis of the information provided and the risks forecast determine under which category of work the proposals fall and whether to endorse the work proposed;

(m) to maintain records of approved project proposals for laboratory genetic manipulation work (including notification for project exemption) and the assessments;

(n) to forward summaries of all project proposals submitted for IBC notification, and the assessments to the Technical Advisory Committee for records and information or for review and recommendation in the case of proposals for Risk Category 2 and 3 work;

(o) to undertake risk assessment, in cooperation with the research teams as necessary, to determine the appropriate containment and biosafety conditions, operating procedures and emergency safeguards for Risk Category 2 and 3 genetic manipulation work, and for the housing, storage or movement of regulated material and also the waste;

(p) to prepare, in conjunction with the research teams, specific contingency plans after undertaking risk assessments and reviewing project proposals;

(q) to enforce with particular emphasis on Risk Category 3 work, all recommendations, and ensure that the conditions of National Biosafety Committee have been acknowledged and promptly addressed;

(r) to inspect and certify, before use in genetic manipulation work. C1 level laboratories, conventional animal houses, PH 1 plant glass houses, and quarantine and medical facilitates for infected animals; and

(s) to monitor and assess the containment features of and the working conditions within all laboratories, plant glass houses and animal houses supporting the institution's work, to ensure that the various facilities are maintained at the standards and requirements delineated in Appendices 4 through 11 of biosafety guidelines.

5.3.5. Responsibilities

To ensure that laboratory genetic manipulation work within the institution conforms to the regulations within these Guidelines, the IBC must address the following tasks:

A. Assess all projects referred to the committee, and on the basis of the information provided and the risks forecast determine under which category of work the proposals fall and whether to endorse the work proposed.
B. Maintain records of approved project proposals for laboratory genetic manipulation work (including notification for project exemption) and the committee's assessments.
C. Forward summaries of all project proposals submitted for IBC notification, and the committee's assessments, to the NBC for records and information -- or for review and recommendation in the case of proposals for Risk Category 2&3 work.
D. Undertake risk assessment, in cooperation with the research teams as necessary, to determine the appropriate containment and biosafety conditions, operating procedures and emergency safeguards for Risk Category 2 and 3 genetic manipulation work, and for the housing, storage or movement of regulated

material and also the waste.

E. Prepare, in conjunction with the research teams, specific contingency plans after undertaking risk assessments and reviewing project proposals.

F. With particular emphasis on Risk Category 3 work, enforce all recommendations, and ensure that NBC and committee comments have been acknowledged and promptly addressed.

H. Inspect and certify, before use in genetic manipulation work, C1 levellaboratories, conventional animal houses, PH1 plant glass houses, and quarantine and medical facilities for infected animals. (The TAC & NBC will be responsible for certification of higher-level laboratories and containment facilities only).

I. Monitor and assay the containment features of, and the working conditions within all laboratories, plant glass houses and animal houses supporting the institution's work, to ensure that the various facilities are maintained at the standards and requirements delineated in Appendices 4 through 11.

5.4. THE BIO-SAFETY OFFICER (BSO)

Institutions and organizations involved in genetic manipulation work should appoint a Bio-Safety Officer to the IBC. Alternatively, institutions affiliated with an IBC yet without services of a BSO may opt to transfer the responsibility of securing a biosafety officer over to thecommittee. For larger institutions contracting the services of multiple BSOs, the NBC requires that one representative shall be designated and shall serve as the contact person. BSO's on leave of absence must arrange for competent replacement to take up the forsaken responsibilities.

To meet the objectives of these Guidelines, BSOs should have considerable experience with pertinent biosafety issues and emergency counter-measures. The BSOs are expected to have undergone rigorous training on biosafeguards in order to participate in the training and instruction of personnel, to review (in conjunction with the IBC, and on a regular basis) operating procedures and biosafety records, and to assay the integrity of containment facilities and safety equipment/utilities.

The BSO and the Chairperson of the IBC shall assume direct advisory positions to the head of the institution on all matters pertaining to risk and biosafety, the health of personnel, contingencies at work and infractions of national guidelines. As with the IBC, the BSO shall set apart time for researchers and for laboratory and field personnel to approach the officer with questions, disputes and concerns.

5.5. PERSONNEL CARE AND MANAGEMENT

Institutions and organizations, contracting personnel for work in genetic engineering and biotechnology must ensure, through the IBC, that all personnel have been instructed on applicable codes of conduct and are conscious of the risks and hazards involved in their line of work. Personnel should receive supplementary training and instruction on laboratory and/or field procedures, emergency safeguards and equipment operations relevant to their line of work periodically. The IBC, the BSO or the project supervisor may administer tests without prior notice to check up the faculties and the caliber of each individual. No one shall be allowed to work under high-hazard or high-risk situations unless they have consistently exhibited good microbiological practice and a requisite understanding of operational routines.

Institutions engaged in microbiological genetic research are strongly encouraged to collect and store baseline serum samples from all personnel at high risk at regular intervals for future reference--in the event of contingencies whereby individuals are overtly or unduly exposed to regulated material and fall sick from unusual or unexplained causes. Institutions should institute a programme to be supervised by the IBC, especially where work involves toxic, pathogenic or infectious determinants. Provisions for serological monitoring, general health surveillance and medical treatment must be given due consideration.

Personnel with questions and concerns regarding any issue of biosafety or operational routines should feel free to approach the BSO or the Chairperson of the IBC, among other authorities.

5.6. ACCIDENTS AND EMERGENCIES

The IBC, in conjunction with the BSO and appropriate divisions of the institution, shall adopt a system for reporting laboratory accidents, occupational hazards and personnel exposures, through to the emergency procedures observed in dealing with such incidents.

In the event the Chairperson of the IBC believe any incident (e.g. deliberate attempt to circumvent these Guidelines) to be of gravity, of solemnity or of the potential for major repercussions to the community or the environment, the Chair should present the deliberations of the committee to both the NBC and the head of the institution. The various authorities may then cooperate in implementing further measures to deal with the problems uncovered, if need be.

5.7. THE PROJECT SUPERVISOR

The project supervisor or head researcher should be a trained scientist with thorough understanding of the codes, regulations, and laws applicable to genetic engineering and biotechnological work and exhibit an appreciation for the biosafety concerns that underlie the need for such provisions.

As the officer-in-charge, much of the responsibilities of the project supervisor rests in the initial stages of originating proposals and obtaining IBC approval, where necessary. For laboratory genetic manipulation work, the project supervisor should assess the nature of the research and determine whether the work proposed falls within the scope of these Guidelines. Uncertainty and doubt should be addressed by submitting a detailed proposal of the experimentalconditions to the IBC for endorsement or clearance before any work is carried out. If work is indeed regulated under these Guidelines, the project supervisor must submit a completed project proposal form (including requests for exempt status) to the supervising IBC for consideration and recommendation, and inform the committee of any notable intents (e.g. plans to import regulated material). Laboratory work may begin after authorization from the IBC. As directed by the IBC, the project supervisor may be required, from time to time, to provide additional details of the research for the various evaluation and monitoring activities of the committee.

The project supervisor should sincerely enforce the provisions and adhere to the intent of these Guidelines through the duration of research work, with special emphasis on the following charges:

A. Submit new project proposals to the IBC for consideration and recommendation before adopting radical operating procedures or substantially changing any parameter of the work (especially approaches to physical and biological containment) which may introduce novel risks, delimit new biosafety levels or warrant change of classification.
B. Establish and maintain working conditions appropriate to the level of biosafety as approved and advised by the IBC and, in the case of Risk Category 2&3 work, in accordance with the recommendations of the NBC.
C. Ensure that students, junior personnel, co-investigators and other persons entering controlled areas realize the nature and degree of the risks involved and have been properly instructed on applicable codes of conduct.
D. Cooperate closely with the IBC and BSO in carrying out various safety tests, for instance, inspections of containment facilities and personnel examinations.
E. Report all personnel developments, including unusual illnesses, to the IBC.
F. Relay to the IBC, details of all contingencies and the emergency procedures instigated to deal with such incidents.

5.8. RELATIONSHIP BETWEEN IBC, TAC & NBC

The guidelines propose that all "no risk" "minimal risk" and "low risk" works including laboratory work and field trials be assessed, evaluated and permission granted by IBC. All category 3 risk work (laboratory & field trials) be evaluated and permission granted by the NBC. All works with no prior history of risk assessment and all requests for deregulation and commercial releases of Genetically Modified Organisms and products thereof, will be decided by NBC, on the evaluation/assessment report of TAC (Figure-1). All application, requests will be submitted to the relevant IBC which will transmit it to the TAC/NBC with its assessment/report for further necessary action. The communication channel will be IBC→TAC →NBC. Further, all clearances granted by IBC and TAC will be considered and decided by NBC. The secretariat of NBC will work as a national depository of all biotechnological projects. All applications and documents submitted to IBC for onward transmission to NBC will go through the relevant ministry to the TAC. The flow-chart is self-explanatory (Figure-2).

FIGURE-1

JURISDICTION OF VARIOUS BODIES
(Containment)

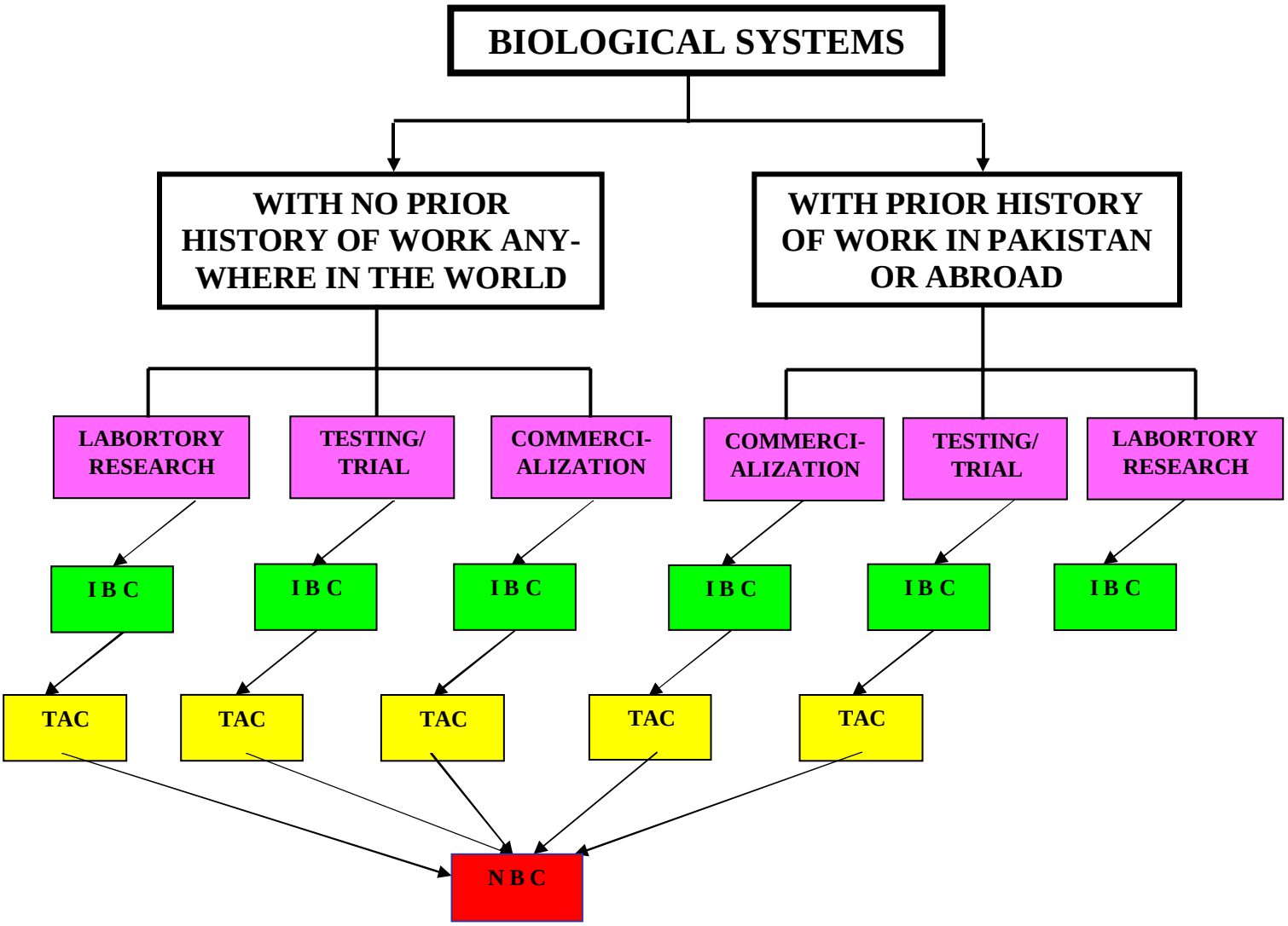
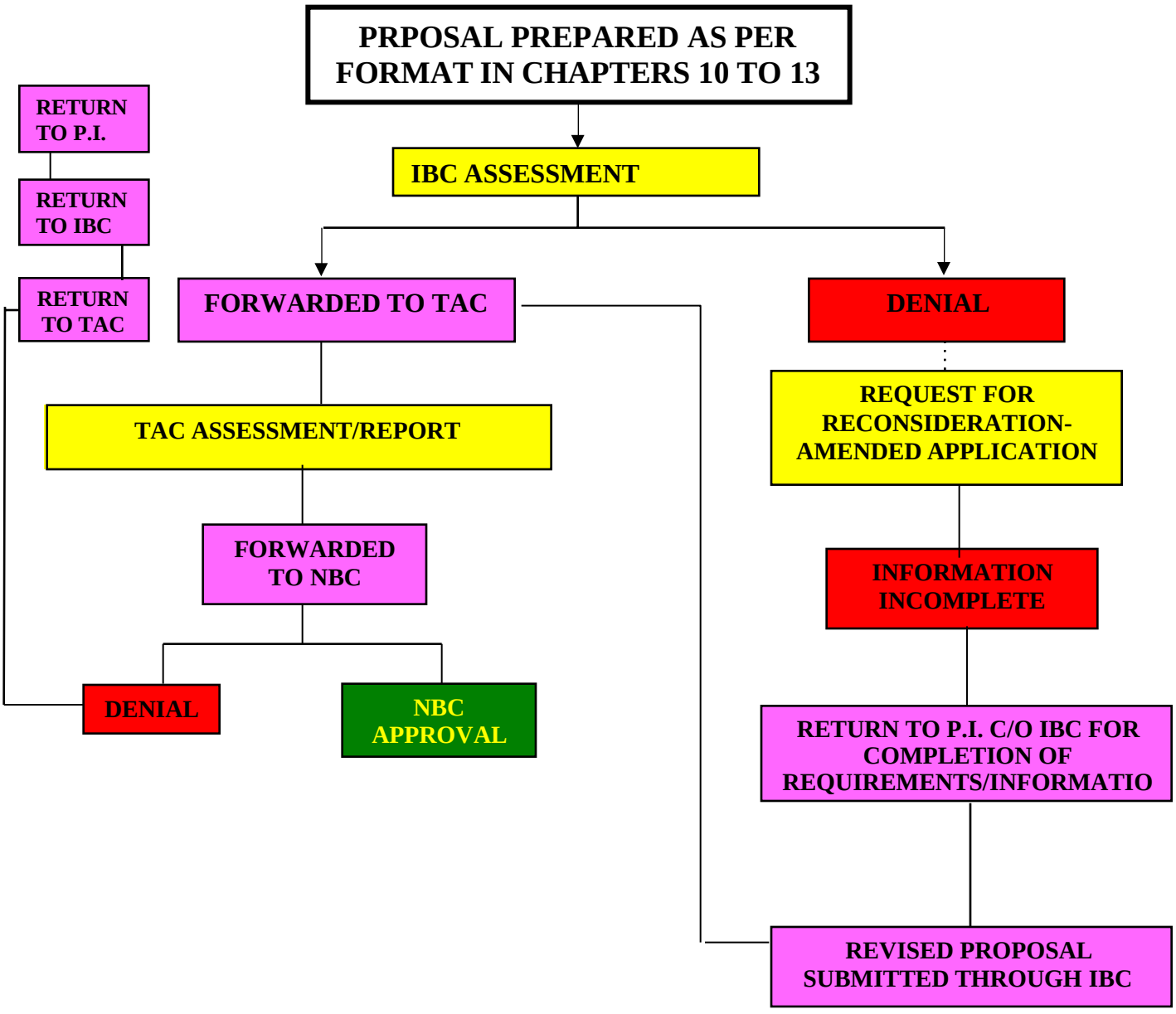


FIGURE-2

PROCESS FLOWCHART FOR REVIEW OF APPLICATIONS FOR PERMISSION TO UNDERTAKE LABORATORY, FIELD TRIALS AND COMMERCIAL RELEASE OF GMO'S AND THEIR PRODUCTS



C H A P T E R 6

SPECIAL REQUIREMENTS FOR SOME LABORATORY GENETIC MANIPULATION WORK

6.1. WORK INVOLVING HAZARDOUS GENETIC FRAGMENTS

Isolating genetic material for laboratory use in host/vector systems may convey various risks and hazards depending, primarily, upon the nature of the genetic material involved. In particular, the following materials are recognized to bear substantial risks and hazards to laboratory personnel:

- DNA coding for various proteins, known or suspected to directly, or indirectly, regulate cellular metabolism, growth or division (e.g. growth factors/hormones and their receptors).
- Genes encoding lethal or virulent toxins.
- All oncogenes, especially those exhibiting high levels of gene expression or linked to gene promoters with high activity in human cells. For a particular DNA fragment, hazards are multiplied with the number of oncogenes encoded.
- Whole viral genomes.
- Fragments of viral genomes which retain the potential to regenerate live viruses.

The principal concern of work with hazardous genetic fragments is that the material involved may penetrate the skin of personnel through cuts and surface wounds or contaminated needles, enter cells and get introduced into tissues. As such, gloves are required, atall times, to avoid skin contact and extreme care must be taken in handling sharp instruments, blades and edges e.g., needles and scalpels.

6.2. USE OF LIVE VIRAL VECTORS

Many vectors in common use are live viruses, developed to achieve unprecedented level of efficiency for the transfer of genetic material into cells. Live viral vectors, however, are often associated with a number of risks and hazards, most are not fully characterized but all are dependent upon the following criteria.

- Host range of the virus.
- Modes of transmission (e.g. air-borne, through body fluids).
- Infectivity and communicability (including epidemiological considerations).
- Opportunities for repeated rounds of infection.
- Natural tendency of viral genetic material to become inserted into host cells and incorporated into host DNA.
- Nature of foreign genetic material inserted (including functions of target genes).

Nearly all proposals for use of live viruses as vectors fall into Risk Category 3, and accordingly, must be sanctioned by the NBC before work may begin.

Note: The following consideration and recommendations are meant to apply to the use of retroviral vectors but may be adapted for genetic manipulation work with other classes of live viral vectors as well).

6.2.1. Retroviral Vectors

The retroviruses developed thus far, have proven to be highly efficient for the transfer of genetic materials into a variety of cells. In principle, retroviral vectors are no different from conventional eukaryotic vectors in that all provide the fundamental regulatory sequences for the control of introduced gene expression.

The majority of retroviral vectors are replication-defective because the target gene(s) substitutes for or displaces a sequence of the genome regulating some important parameter of viral replication or of packaging of viral particles. However, this capability for viral propagation can be restored in cases where the missing genes are provided to the experimental system by the use of specific non-defective helper viruses. Alternatively, helper functions may be stable integrated into one of the chromosomes of a variety of packaging cell lines. Such lines will allow for the regeneration of fully infectious retroviruses but which cannot replicate and are not communicable/transmittable. Either way, the types of cells that may serve as hosts (i.e. that may be infected), depend on the particular helper virus adopted, or helper functions afforded by the packaging cell line chosen.

Retroviruses can be classified in a number of ways, but in the interests of these Guidelines, it is helpful to consider retroviruses on the basis of host range, as infectivity is a major concern of general biosafety considerations.

6.2.2. Classes of Retroviruses According to Host Range

Amphotropic retroviruses	The Amphotropic viruses will grow in the cells of the species from which they were isolated and also in the cells from a wide range of other species.
Ecotropic retroviruses	The ecotropic viruses will grow in the cells of the species from which they were isolated and to a limited or undetectable level, in the cells of other species.
Xenotropic retroviruses	The xenotropic viruses are endogenous to a species but cannot replicate well in that species, generally because of a receptor block. They tend to have a wide range for replication in the cells of heterologous species.

As all the viruses described bear appreciable levels of risk and hazards (highly infectious), extreme caution is warranted regarding the nature of the target gene(s) when working with retroviral vectors. Where the introduced genetic material is itself hazardous, the risks involved are multiplied. In particular, extreme caution must be observed in handling retroviral vectors, capable of infecting and/or propagating themselves in human cells, which incorporate the following hazardous genetic material:

- known oncogenes of viral or cellular origin.
- genes coding for proteins which regulate, or may alter, the growth patterns of mammalian cells or forming the pattern of traits (e.g. growth factors/hormones and their receptors).
- genes encoding molecules whose activity/expression is modulated by the stimulation of growth factor receptors.

- genes coding for known or suspected toxins (e.g. cytotoxic proteins).

As with hazardous genetic material, the principal concern of work with retroviruses is the possibility of viruses entering cells and tissues through surface wounds and broken skin, or by accident. Most of these risks are directed towards those involved in the actual manipulations or working in the immediate vicinity, as retroviral particles are extremely labile and short-lived.

Construction of recombinant retroviruses must comply with basic National Biosafety Guidelines for laboratory genetic manipulation work. Once constructed though, the handling and application of these viruses must likewise proceed in accordance with the relevant supplementary procedures/practices detailed hereinafter (section 7.2.2. through 7.2.5.).

6.2.3. Approach to Non-Human Ecotropic Retroviruses

Non-human ecotropic retroviruses, because of perfectly limited host range, will not grow to any significant level in human cells and are not regarded as dangerous to laboratory personnel. Simply the adoption of good tissue culture practices is enough to ensure a satisfactory laboratory biosafety environment. Nevertheless, it is strongly recommended that various fluids, wastes and by-products from experimental cell cultures and organisms be thoroughly decontaminated/inactivated before disposal, to prevent such ecotropic viruses from fortuitously infecting natural cell lines.

6.2.4. Approach to Defective Retroviruses with Human Cell Host Range

Inherently, retroviruses with human cell host range are amphotrophic, xenotropic or human ecotropic viruses. All may bear substantial risks to laboratory personnel if associated with hazardous gene sequences. Even where the risks are reduced by the use of defective retroviruses, investigators must strictly abide by good virological and tissue culture practices, with special emphasis on the following precautions:

A. Perform all manipulations and handle all cultures of viable retroviruses in Class II biological safety cabinets or equivalent primary containment units. Only one individual may use a biological safety cabinet at any one time.

B. Handle cultures of amphotropic retroviruses or of cells, infected with amphotropic retroviruses in 'double-hulled' units (e.g. plates with inverted lids) to trap accidental spills.

C. Store amphotropic viral stock in an appropriately designed and isolated section of the freezer and store ampoules of frozen amphotropic cell lines in a separate section of the liquid nitrogen tank. All such divisions need to be properly labeled/tagged. Laboratory personnel are responsible for the care of amphotropic viral stock and cell lines.

D. Separately incubate tissue cultures infected with amphotropic retroviruses from tissue cultures infected with ecotropic retroviruses.

F. Take special care to avoid skin contact with retroviruses and contaminated material. Wear over-all and rubber gloves whenever operating on retroviruses carrying hazardous genetic materials, as described in section 7.2.1.

G. Wear molded surgical masks or respirators, as necessary.

H. Keep the use of sharp and pointed instruments (e.g. hypodermic needles, blades, syringes) to a minimum and take special care to avoid auto-inoculation. If practical alternatives exist, never use sharp instruments on amphotropic retroviruses and cell lines. If not, these instruments must be discarded into separate biological disposal receptacles before sterilization.

I. Use only mechanical pipetting devices; mouth pipetting is prohibited.

J. Decontaminate all pipettes and glassware after use, by autoclaving or using chlorine preparations.

K. Autoclave effluents, wastes and by-products before disposal; exhaust gases from primary containment units and hoods must be treated by high-efficiency filters before release.

L. Wash down contaminated surfaces with chlorine disinfectants and immediately decontaminate spills. Project supervisors have the responsibility for determining the appropriate disinfectant to be used in any given situation, considering the nature of contaminant and of work surface contaminated. As a standard practice, glutaraldehyde disinfectants should be used in Class II biological safety cabinets.

M. Wipe down work surfaces with chlorine or iodide preparations, glutaraldehyde disinfectants or other disinfectants, as suitable, after each session and before ultraviolet sterilization.

Both the project supervisor and the IBC must be actively involved in monitoring biosafety conditions within the laboratory. Before work with genetically modified or recombinant retroviruses, it is critical that a serum sample be kept for future reference and health surveillance, in case of occupational hazards that lead to overt or undue exposures. During the conduct of research, it would be helpful to maintain a central register, providing a chronological inventory of work with, and storage/disposal of, retroviral vectors and cell lines. Above all, and at all times, insurance must be made that only highly trained and proficient personnel operate on infectious retroviruses with human cell host range.

Work with amphotropic retroviruses warrant exceptional caution. Hoods used in manipulations of amphotropic retroviruses must be meticulously wiped down with chlorine disinfectants, and subsequently ultraviolet-sterilized, before use with other non-amphotropic retroviral stains. Where recombinant amphotropic retroviruses are to be propagated in human cells, there is a need to show that cultures are free of human retroviruses. Nonetheless, cell cultures from laboratory personnel and their immediate relatives must never be infected under any circumstances, with any class of retrovirus.

6.2.5. Handling of Animals Infected with Non-Human Ecotropic Retroviruses

As non-human ecotropic retroviruses bear negligible hazards towards laboratory personnel, the adoption of good animal handling practices is considered adequate for work with infected animals. As a standard procedure, wastes, secretions and by-products from experimental animals must be thoroughly decontaminated before disposal, to avoid inadvertent infection of wild relatives and natural variants.

Infected and uninfected animals must be kept in separate cages (but may be held in the same room) to minimize the opportunity for repeated rounds of infection.

6.2.6. Handling of Animals Infected with Retroviruses with Human Cell Host Range

The infectious retroviruses, with human cell host range, need stricter precautions to those precedent. As basic requirements, infected species must be kept in separate, appropriately labeled cages (and apart from uninfected animals) and all wastes, secretions and by-products from experimental animals must be thoroughly decontaminated/inactivated before disposal. In the laboratory, work benches should be layered with protective paper, which needs to be replaced regularly.

On top of good animal handling practices, stringent arrangements must be made to enhance physical containment, preventing escape and spread of infection. Extreme caution must be observed when dealing directly with infected animals to avoid being bitten, clawed or scratched--in performing operations, gloves and cover-all must be worn to avoid skin contact with animal tissues and body fluids.

In spite of all safeguards, handling animals, infected with retroviruses with human cell host range, guarantees much risk. Only highly trained and proficient personnel, under supervision of the project supervisor, should be allowed to operate on infected animals.

6.3. WORK WITH WHOLE PLANTS

Recognizing the relative facility with which many plants propagate, disperse and outcross, field testing work on plants requires for submission "Supplementary Information" in addition to the standard project proposal. The supplementary information will include details of the auto-ecology of relevant noxious weeds and plans for cultivation of transgenic species. The additional details provided in supplementary information will be reviewed by the IBC and shall constitute the framework for committee recommendations regarding containment, disposal and safeguards.

Criteria to be followed for field testing of the transgenic plants that do not meet the criteria in Section 3.2.1.

A. Maintain at least the minimum isolation distance recommended for raising "Foundation Seed" all around the transgenic plants. The distance will vary from crop to crop. In the isolation distance a non-compatible crop can be grown. Alternatively, the researcher could bag flowers to prevent pollen flows. In the case where seeds are not needed, remove flowers or destroy plants before flowering.

B. Beyond the isolation distance, grow a few rows of nontrangenic plants of the same crop to serve as a pollen trap for insect pollinated crops such as cotton.

C. Analyze the seed progeny from the plants used as pollen trap for accessing pollen escapes and verifying the effectiveness of the isolation distance. The experiments conducted to monitor pollen escape into compatible border row plants should be maintained that will be useful for risk assessment.

D. Pre-release tests of genetically engineered organisms in agricultural applications should include elucidation of genetic markers, host range, requirements for vegetative growth, persistence and stability in small plots and experimental field trail.

6.3.1. Post Harvest Handling of the Transgenic Plants

A. Destroy by burning all the left over vegetative parts and left over seeds, if not needed for future experiments.
B. Leave the land fallow next year and destroy the plants, if any emerging from the seeds of last year in the soil.
C. Soil samples in experiments under controlled containment conditions should be tested for the absence of viable cells before disposal into the environment.

The relevant biosafety committee initially may clear a project for limited field trials which may be enough to have data for assessing the environmental impact in case it is necessary.Where such environmental impact studies are not warranted, the committee may give permissionfor large scale trials.

6.4. ENGINEERING OF TRANSGENIC ANIMALS

All genetic manipulation work on live animals, fertilized oocytes or early embryos, to the extent that a novel organism results, falls into Risk Category 2. Nevertheless, proposals for such work that entails the introduction of viral genetic material or whole viral genomes into zygotes and embryos official IBC authorization must be reviewed by the NBC.

6.4.1. References

The following references are essential to all investigators and IBCs involved in genetic manipulation work with transgenic animals:

- USDA/APHIS 1999, http://www.aphis.usda.gov
- OECD, 1999. http://www.oecd.org/biobin
- ISAAA, 1995. Food safety evaluation for the products of biotechnology. The ISAAA food safety initiative, ISAAA, Ithacca, N.Y.
- Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 1990 (prepared by the NH&MRC, the Common wealth Scientific and Industrial Research Organization, and the Australian Agricultural Council).
- Australian Standards 2243-1991 Safety in Laboratories: Part 3-Microbiology (basic standards for animal houses).
- Procedures for Assessment of the Planned Release of Recombinant DNA Organisms, 1987 (available from the GMAC Secretariat, GPO BOX 2183 Canberra ACT 2601, Australia).

Other key references and recommended reading are listed under Appendix-1.

6.4.2. Supplementary Information

If the proposed work involves genetic modification of animals, the project supervisor must prepare a "Supplementary Information" for the Engineering of Transgenic Animals, addressing the various concerns in the design of animal containment facilities as are outlined in Appendices 10 and 11. Append all supplementary information to the Project Proposal Form for Assessment by the supervising IBC. The supplementary information will guide the IBC in reviewing the design of animal housing facilities, as appropriate containment measures will vary with different experimental and biological systems. Further, the project supervisor must ensure that no work shall start prior to receiving IBC endorsement.

6.4.3. IBC Assessment for Proposals for Engineering Animals

While considering a proposal for the genetic modification of animals, the IBC must inspect all animal containment facilities to be used, in addition to reviewing the various written forms submitted. Where work raises moral questions and concerns, the IBC should consult religious scholars and other personnel well-versed with bioethics.

If the IBC endorses the proposal, it must immediately forward with copies of relevant assessments and the original submissions to the NBC. Only after receipt of NBC approval and complete reassessment of proposals, may the IBC give authorization for research work to begin. If final endorsement is conditional upon the adoption of additional provisions, send a copy of the terms of approval--under heading of the IBC Form for Assessment of a Proposal to carry out Laboratory Genetic Manipulation Work--to the project supervisor.

6.4.4. Important Points:

Experimental animals may range from small, laboratory species (e.g. fruit flies, mice, rabbits) to large, domesticated species or livestock (e.g. sheep and cattle). As such, the scale of housing requirements may vary considerably from one proposal to another, although in principle, microbiological safety considerations would remain similar. On a related note, in case of the considerable scale involved NBC guidance may be required of work where large numbers of transgenic animals are to be handled together.

The outcome of any introduction of novel genetic materials into the genome of animals is never entirely predictable. No matter how precise the method of introduction, the desired gene expression cannot be guaranteed, and transgenic animals may instead exhibit incidental genetic and phenotypic variability (depending on a number of factors, particularly the stability of incorporated transgenes, their effects on neighboring operons and related changes in biosynthetic and energy demands). The method for introduction of donor DNA can likewise bear undesirable side-effects. Researchers must refer to the IBC, those cases where transgenic animals harbor infectious agents that are transmissible to other animals or humans within the same holding facility. Concerns regarding possible/likely routes of transmission must be removed. Ideally, accommodations for experimental animals in laboratory genetic manipulation work, need to be physically separated from facilities supporting other activities, such as animal nurseries and animal quarantine areas. Where there is to be concurrent use of a transgenic animal house, researchers must adopt suitable precautions to avoid interference with the proposed work.

6.5. GENETIC ENGINEERING OF INFECTIOUS ANIMALS

Genetic engineering of infectious animals -- including transgenic animals produced by the introduction of genes from infectious agents, and animals which incorporate genes encoding infectious particles--need special housing considerations addressing the particular risksconveyed. For the most part, physical containment precautions for laboratory work with these animals would follow the general requirements of C1A or C2A animal houses (Appendices 10 and 11 respectively), as appropriate to the level of concern. However, higher levels of animal containment will be warranted and investigators must refer such instances to the NBC for consultation with the committee regarding special conditions to be adopted. Ideally, animal houses must be integrated with the main laboratory to facilitate transport, observation and experimentation.

As a general principle, the biological and physical containment consideration, for such in vivo work are comparable to those for work with infectious agents in vitro.

Note: As a reference, the housing of infectious animals must observe the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, NH&MRC/CSIRO/AAC, 1990.

C H A P T E R 7

MOVEMENT OF REGULATED MATERIALS

7.1. MOVEMENT OF REGULATED MATERIALS WITHIN OR BETWEEN INSTITUTIONS

Extreme care must always be observed in moving regulated materials within and between institutions. Genetically modified materials (transgenic plant and animal materials, e.g. cuttings, seeds, eggs, tissue samples) must be transported in securely sealed, double-containment units, each comprising a primary container for holding the organism(s) or culture/preparation, enclosedin a durable, secondary container which may be readily decontaminated. These units should ideally be placed within sturdy outer shipping containers-secondly packaged and appropriately labeled and addressed to facilitate inspection. That will allow swift delivery to the intended destination and ensure that relevant authorities are contacted in case of emergencies. Movement of wastes and by-products from genetic engineering practices require comparable packaging and container specifications.

7.1.1. Transport of genetically modified microorganisms

The essential restriction on the transport of viable genetically modified microorganisms allows for only those arrangements which ensure that transgenic species in transit will not be harmful to the community or the environment if the packaging or container integrity becomes compromised enroute. Species recognized as benign to the humans and the environment may be transported within basic packaging and container requirements. Microorganisms, pathogenic, infectious, or hazardous to the environment in one way or another, shall only be moved provided that the mode of transport offers suitable decontamination features.

7.1.2. Transport of transgenic plants and animals

The transport of transgenic plants and animals should be supervised by someone skilled and with considerable experience. He/She should be able to handle transgenic species and initiate population control programmes in the event of unforeseen contingencies. Stringent and selective containment must be adopted as necessary, taking into account, to the greatest extent possible, various contingencies which may be encountered so as to minimize the potential for escape and to prevent transgenic species from inbreeding freely with and becoming established in wild populations. Proper arrangements should be made to identify and account for individual animals, plants, or containers in transit.

As to the transport of transgenic plants, it is recommended that whole plants be netted and deflowered beforehand and that plants which have set seed not be moved.

7.2. DISTRIBUTION AND RECEIPT OF GENETICALLY MANIPULATED MATERIALS

Researchers distributing genetically manipulated materials to scientists and institutions, local or abroad must provide recipients with reviews of the physico-chemical and biological containment measures, safety precautions, and any special guidelines for work involving the material circulated. Researchers should also detail the origin of regulated material distributed, to serve as terms of reference for each recipient. In the event of a local beneficiary without previous links to or background in genetic engineering and biotechnology, the distributor has a further responsibility to ensure that the recipient is made aware of the national guidelines regulating work in this discipline.

Distribution and receipt of genetically manipulated material must be reported beforehand to the appropriate director of the institution for legal purposes.

7.3. IMPORT AND EXPORT

Individuals, who wish to import viable microorganisms, plants or animals, genetically modified or constructed, must proceed in accordance with the relevant guidelines presented herein. They are strongly encouraged to consult with the IBC regarding the specifics of their intent.Import of live or whole organisms of another nature is regulated directly by the various orders and enactments presented under Pakistani Law.

On the other hand, international postage or export of regulated material must strictly comply with the revised provisions and requirements of The Non-infectious and the Infectious Perishable Biological Substances Services as agreed to by the International Postal Union (IPU).

Import and export of pathogens must observe the terms of the Quarantine Laws. Import of transgenic plants of any form must observe the terms of Plant Quarantine Act.

7.4. CONTROLLED MOVEMENT OF GMO'S AND PRODUCTS THEREOF

In order to eliminate risks/hazards that may be associated with uncontrolled movement of GMOs and products thereof, it is proposed to regulate the movement of such materials from one lab to the other or from the lab to the field within the country or due to importation for commercial and/or research purposes. All responsible researchers desirous of moving regulated materials must therefore, seek permission from the relevant IBC on a specific format given on Pages 39 to 44. The researchers must submit two sets of applications, one of which will be retained by the IBC and the other returned to the applicant with permission of the IBC with or without provisos. Separate forms should be used for the movement involving importation from another country or moving mammalian cell line or plant materials.

7.5. IBC ARRANGEMENTS

The IBC may impose additional security precautions as it sees fit, to address the specific risks and concerns of any transport at hand. Furthermore, the IBC may feel obligated to personally survey and inspect the preparations for transport of transgenic species, to ascertain whether standard requirements and additional precautions, if any, are being attended to.

7.6. The above is in addition to the terms and conditions of biosafety agreed as a party to the Open-ended Adhoc Working Group on Biosafety.

National Bio-safety Committee (NBC)
Pakistan Environmental Protection Agency
Ministry of Climate Change &
Environmental Coordination,
Government of Pakistan
Islamabad.

Application for Permission for the Movement of Regulated Materials

1 Name and full professional address of Applicant.	2 Permission Requested	3 This Request is)
	Limited In-Country Movement [ ] Limited - Importation [ ]	New [ ] Renewal [ ] Supplemental [ ]
Tel: **Fax:** **Email**	5 Permission Requested ("X" one) Mail [ ] Baggage or handcarried [ ] Common carrier [ ] By Whom _____	

6 Give the following (if applicable) if more space is needed attach additional sheet)				
	Scientific Name	Common Name	Trade Name	Other Designation
a. Donor Organism				
b. Recipient Organism				
c. Vector or Vector Agent				
d. Regulated Organism or Product				
e. If product, list names of constituents				

7 Quantity of regulated article to be introduced and proposed schedule and number of introductions	8 Date (or inclusive dates of period) of importation or in country movement

9 Country or point of origin of the regulated article	10 Port of arrival/destination movement or specific location.

11 Any biological material (e.g. culture medium, or host material) accompanying the regulated article during movement.

12 State why you believe the organism or product does not come within the definition of a regulated article.

13 **Certificate: I hereby certify that the information in the application and all attachments is complete and accurate to the best of my knowledge and belief**

14 Signature of Applicant	15 Printed name and title	16 Date

IBC No.

National Bio-safety Committee (NBC)
Pakistan Environmental Protection Agency
Ministry of Climate Change &
Environmental Coordination,
Government of Pakistan
Islamabad.

Application for Permission to Import or Transport Organisms or Vectors

1 Mode of transportation (Please circle).	2 Port of Entry
AIR SEA LAND ANY	

IMPORTER: (Name, complete address, telephone and fax number).	SHIPPER: (Name and address of foreign producer)
Tel: **Fax:** **Email**	**Tel:** **Fax:** **Email**

5 DESCRIPTION (material, country of origin, animal source, recombinant system & genetic inserts conditions of imported preparation, antibody immunogens etc. (COMPLETE VS FORM 16-7 for all cultures & their.

6 Quantity and frequency of importations (estimate)

7 Proposed use of material expected completion date, and final disposition (method)

8 Description of applicants facilities and equipment for handling

9 Qualifications of technical personnel working with this material (if applicable)

10 Treatment of material prior to importation (processing/purification methods, treatments, disease safeguard, etc.)

11 Work objectives, proposed plan or work, and additional pertinent information (animal model, use of derivatives, etc.)

12 Pertinent Published Paper/Abstract regarding material to be imported – attach copy, if available

13 Certificate: I hereby certify that this material will be used in accordance with all restrictions and precautions as may be specified in the permit

14. Signature of Applicant	15. Printed name and title	16. Date

IBC No.

National Bio-safety Committee (NBC)
Pakistan Environmental Protection Agency
Ministry of Climate Change &
Environmental Coordination,
Government of Pakistan
Islamabad.

Application for Permission to Import or Transport Plants or Plant Products

1	PLANTS OR PLANT PRODUCTS TO BE IMPORTED	
COUNTRY OF ORIGIN Make separate entry for each Country).	QUANTITY AND NAME OF PLANTS OR PLANT PRODUCTS Scientific (*Botanical*) or English names must be included (*colloquial names are* not acceptable) List whether seeds, bulbs,plants, cuttings, cut flowers, fruits, etc. Indicate whether for planting, consumption, or other purpose.	PAKISTANI PORT OR PORTS OF ARRIVAL

Use additional pages if more space is needed and "X" box ϒ

2	MEANS OF IMPORTATION	Air Mail or Parcel Post [ ] Air Freight [ ] Car [ ] Surface Mail or Parcel Post [ ] Truck, Rail, or Ship [ ] Baggage [ ]

3	Approximate Date of Departure For Pakistan	Arrival Date	4	Are Other Importation Contemplated Within the Next Two Years

ANSWER 6, 7, AND 8 ONLY IF IMPORTED MATERIAL WILL BE RESHIPPED TO ANOTHER COUNTRY

5	Reshipment will be by	AIR [ ]	WATER [ ]

6	Port of exit from Pakistan	7	Country of final destination.

8	NAME AND ADDRESS OF APPLICANT

9	**Signature of Applicant**	**Telephone**	**Telephone No.**	**Email Address**

ENCLOSURES		ENCLOSED ("X")	IF PREVIOUSLY SUBMITTED LIST DATE & PERMIT NO.
a	Names, addresses, and telephone number of the persons who developed and/or supplied the regulated article.		
b.	A description of the anticipated or actual expression of the altered genetic material in the regulated article and how that expression differs from the expression in the non-modified parental organism (e.g., morphological or structural characteristics, physiological activities and processes, number of copies of inserted genetic material and the physical state of this material inside the recipient organism (integrated or extrachromosomal), products and secretions, growth characteristics).		
c.	A detailed description of the molecular biology of the system (e.g., donor-recipient-vector) which is or will be used to produce the regulated article.		
d.	Country and locality where the donor organism, recipient organism, and vector or vector agent were collected, developed, and produced.		
e.	A detailed description of the purpose of the introduction of the regulated article including a detailed description of the proposed experimental and/or production design.		
f.	A detailed description of the processes, procedures, and safeguards which have been used or will be used in the country of origin and in Pakistan to prevent contamination, release, and dissemination in the production of the donor organism; recipient organism; vector or vector agent; constituents of each regulated article which is a product; and regulated article.		
g.	A detailed description of the intended destination (including final and all intermediate destinations), uses,and/or distribution of the regulated article (e.g., greenhouses, laboratory, or growth chamber location; field trial location, pilot project location; production, propagation, and manufacture location; proposed sale and distribution location).		
h.	A detailed description of the procedures, processes, and safeguards which will be used to prevent escape and dissemination of the regulated article at each of the intended destinations.		
i.	A detailed description of the proposed method of final disposition of the regulated article.		

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Pakistan Environmental Protection Agency
Ministry of Climate Change &
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Government of Pakistan
Islamabad.

Application for Permission to Import or Transport Mammalian Cell Line

1	Cell line or reference No.	2	Country of origin of cell line

3	Cell line passage history	4	For hybridoma specify immunogen & fusion partners

5	For others specify origin or derivation (e.g. EBV transformation)

6	Identify Media Nutritive Factors (Protein/nitrogen sources used and/or added). Specify country of origin particularly origin (include species) supplements or nitrogen sources.

7	Country or origin and source of enzymes used (e.g. trypsin).

8	Name of any animal viruses studied in the lab where the cell line originates

9	Address of lab where material originated (if different from shipping lab)

10.	If recombinant specify genetic insert.

11	Potential use of imported cells or products (use of derivatives or extracts, immunogen for antibodies produced and production method

18	Name & Signature of applicant
	Date / /

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National Bio-safety Committee (NBC)
Pakistan Environmental Protection Agency
Ministry of Climate Change &
Environmental Coordination,
Government of Pakistan
Islamabad.

IBC Permission

For Movement of regulated materials	To Import or Transport Plant or Plant Products	To Import or Transport Organisms or Vector	To Import or Transport Mammalian Cell Line

1 IBC Review).

2 Specific Comments

3 Permission [ ] with Provisos [ ]

4 Signature of IBC Secretary.

Date / /

C H A P T E R 8

LEGAL FRAMEWORK GOVERNING IMPLEMENTATION OF BIOSAFETY RULES/ NATIONAL BIOSAFETY GUIDELINES

1. The National Biosafety Guidelines have been prepared to support implementation of the Pakistan Biosafety Rules 2005

2. The Pakistan Biosafety Rules 2005/National Biosafety Guidelines give the legal authority to IBC and NBC to monitoring/implementation bodies (namely IBC TAC & NBC), the necessary legal powers to discharge their approved functions and responsibilities, as contained inthe said guidelines/as mentioned in the stated guidelines.

3. All IBCs of the non-governmental/private organizations will, in addition to, prescribed composition of IBC, will include two nominees of the NBC.

C H A P T E R 9

FRAMEWORK OF THE VARIOUS PROPOSALS/APPLICATIONS

The principal goal of adoption of biosafety guidelines is to discern as far as possible, the potential for harm to the environment and/or human beings, stemming from the uses and applications of Genetically Modified Organisms and products thereof. While biosafety itself presents a goal that is not fully achievable, strict compliance with biosafety guidelines detailed in the proceeding pages can help to minimize the potential for harm. It is, therefore, mandatory that all principal investigators shall prepare project proposals for evaluation by relevant bodies. Pages-27 & 28 of these guidelines give a "process flow-chart" for assessment of all project proposals/applications. Accordingly, all proposals/applications are, in the first place submitted to the Institutional Biosafety Committee. Based on the information provided in and of risks/concerns that may be inferred from the information in the proposals, the IBC shall classify the project as risk Category 1, 2 or 3.

The IBC will either approve the proposal with or without provisos, or return it to the proponent for more information. If the risk category is beyond its jurisdiction, the IBC will forward the proposal to TAC. In all cases, the proponent will receive information regarding the status of the project in a maximum of 30 days. The TAC will, likewise, process the proposal/application for approval or review and forward it to NBC and the process will not take more than 30 days. If the project is rated as "high-risk", the NBC will need up to three months to process the proposal/application. If the proponent does not hear from the relevant bodies within the stipulated time, it would imply that there are no serious concerns regarding the associated risks and therefore, the proposal should be assumed to have met approval of the relevant Approval Granting Body.

To ensure uniformity in formulation of project proposals/applications as well as to facilitate/streamline the process of assessment by various committees/bodies, a specific formathas been designed. Accordingly, all proposals have been divided, on the basis of nature of work, into three groups:

A. For laboratory genetic manipulation of microbes/plants/animals.
B. For regulated release (field trial) of Genetically Modified Organisms and products thereof generated as a result of activity under “A”.
C. For permission to deregulate the regulation of GMOs and products thereof generated as a result of activities under “A” and field tested under “B” above i.e. commercialization of genetically engineered organisms and/or products thereof.

A specific format for proposals/applications seeking permission of work done under “A”, “B” and “C” in Chapters 10-13. Attach NBC Cover Sheet page to your proposal/application and submit 3 copies to IBC, follow all instructions/guidelines in Chapters 10-13.

9.1. SUBMISSION OF PROPOSALS

A proposal must be submitted to the IBC responsible for the laboratories where the work is to be carried out before work commences for all activities involving genetic manipulation of microbes, plants, or animals. Where the work is carried out in more than one organization, all IBCs must be informed and reported in the proposal. The project supervisor will submit three completed project proposals to the supervising IBC which shall forward two copies to the NBC for information/guidance/approval and retain one copy for records and reference.

The IBC will assign each proposal a 10-digit number; check the information provided regarding the proposed biological system, the physical facilities to be used and the details of members of the project team. It will then make its assessment of the proposal with regard to the proposed level of physical containment, and adequacy of the experience of the members of the team for carrying out the proposed work.

9.2. APPROVAL OF PROPOSALS AND COMMENCEMENT OF WORK

Work assessed as low to medium risk may commence after a proposal has been assessed and approved by the IBC. Work must be conducted as recommended by the IBC. Work assessed as high risk by the IBC must not commence without the specific approval of the NBCas the case may be.

9.3. CONDUCT OF WORK

The Principal Investigator must ensure that the directions of the IBC and NBC are complied with, and that the physical containment requirements and procedures are met during the course of the work.

9.4. CONFIDENTIAL INFORMATION

Applicants who wish to restrict information provided in the proposal/application should mark the relevant section as "Commercial-in-Confidence".

C H A P T E R 10

FORMAT OF PROJECT PROPOSAL FOR PERMISSION TO UNDERTAKE LABORATORY GENETIC MANIPULATION WORK

IBC No.

National Bio-safety Committee (NBC)
Pakistan Environmental Protection Agency
Ministry of Climate Change &
Environmental Coordination,
Government of Pakistan
Islamabad.

Cover Sheet

Project Proposal for Permission to Undertake Laboratory Genetic Manipulation Work

1	Name and full professional address of Principal Investigator submitting proposal.	2	Name(s) of other Principal Investigators responsible for the project. Please give their professional addresses if different from that in '1'.
	Tel: **Fax:** **Email**		**Tel:** **Fax:** **Email:**

3	Title of Project.

4	Intended date of commencement
	Expected date of completion.

5	Into which category does this work fall?	Category 1 [ ]	Category 2 [ ]	Category 3 [ ]

6	Main Goals

7	Quantify goals requiring approval
	i) ii) iii) iv) v)

8	Signature of Principal Investigator submitting this proposal.
	Date / /

Section A - Introduction

9. Project Title

- Rationale of Studies

10. Project Objectives

- Overall Objective
- Specific Objectives
  - i)
  - ii)
  - iii)
- Objectives during 1st Year
- Time Schedule

Objective-1
Objective-2
Objective-3
.....

11. Intended Date of Commencement; ....…….....
Expected Date of Completion. .....……..

12. Intended Classification
[ ] Category 1 [ ] Category 2 [ ] Category 3

13. Any special precautions/safeguards to be adopted.

Section B - Materials and Methods

14. Details of the Biological System.

A. The origin of donor DNA. Donor organism's geographical distribution, natural habitat, natural predators etc.
B. Characteristics of the donor DNA.
C. Description of Host Organisms or Tissues.
D. Description of Vectors or Methods for transfer of donor DNA into the Host. Degree of relatedness between host and donor.
E. Characteristics of the Host/Vector System(s), as applicable.

- Predicted Stability of Introduced Genetic Traits.
- Identification Characteristics or Markers.

F. Auto-Ecology of each host/vector system

- Viability in Open Environment.
- Possibilities of Crossing to Related and Other Species.

15. History of Prior Work with Components of the Biological System.

16. Laboratories and Facilities where work will be conducted (for each investigator)

- Complete Address of location of the laboratory in which work to be Conducted.
- Level of Physical Containment (Biosafety).
  [ ] C1 [ ] C2 [ ] C3 [ ] C1A [ ] C2A
  [ ] PH1 [ ] PH2 [ ] PH3.
  [ ] Other (please specify)
- Permission for use of facility (if outside worker).

17. Additional information that will be helpful in risk assessment of the Project.

Section C - Personnel Involved with Research Work Proposed

18. Details of Personnel.

- Name, Qualification and Experience.
- Responsibilities and Duties.
- Medical History.

Supplementary Information only for Work on Whole Plants

19. Details of the Biological System

A. Are the experimental plants noxious weeds
[ ] Yes [ ] No
If yes, elaborate on the ecological content:

- Reproductive Cycle and Evolutionary Potential.
- Dispersal, Proliferation and Persistence in Open Environments.
- Factors which might limit growth, reproduction, and survival.
- Natural Crossing Possibilities to wild populations.
- Noxious Characteristics.

B. Are the experimental plants closely related to noxious weeds?
[ ] Yes [ ] No

If yes, indicate species/strains/natural variants; and elaborate on the ecological context of each:

- Reproductive Cycle and Evolutionary Potential.
- Dispersal, Proliferation and Persistence in Natural Habitats.
- Factors which might limit growth, reproduction, and survival.
- Natural Crossing Possibilities (particularly to experimental plants).
- Noxious Characteristics.

C. Are microorganisms involved in these studies. If so, are they harmful to humans, plants, or animals?

[ ] Yes [ ] No

If yes, elaborate on the harmful agent (e.g. pathogenic or infectious determinant? toxic substance?) and the known or likely modes of transmission.

Indicate any potential to cause epidemics.

20. Further Details of the Methodology.

A. Substrate for use in cultivation?

[ ] Soil
[ ] Soil Substitute (specify)

Describe the sterilization procedures.

B. Plans for Cultivation of Genetically Manipulated Plants.

- Developmental Stage(s) Targeted and Intentions to Breed.
- Arrangements for containment of plants and plant materials (spores, seeds, pollen, vegetative materials).
- Arrangements for disposal of plants and plant materials; wastes and by-products which may contain viable plant materials.

21. Details of equipment and facilities for use in cultivation.

22. Any additional information relevant to the assessment of this work.

Supplementary Information only for Work on Transgenic Animals

23. Details of the Experimental Animals.

A. Nomenclature.

- Class, scientific name, and strain.
- Indicate whether a local or an exotic species.

B. Number of Experimental Animals.
(schedule of the approx. number of animals to be handled each year and at any one time).

C. Developmental Stages Targeted.

(stages in life cycle involved, for example, "embryo only" or "through reproductive age").

24. Details of the Genetic Manipulation.

A. Does genetic manipulation involve [ ] germline or [ ] somatic cells?
B. Characteristics of donor DNA.

- Origin and construct (with reference to biological source(s) and to proposal(s) covering preparation of the DNA).

- Characterization and history of prior use in genetic manipulation (with references of published work).
- Physiological traits intended to be conferred (e.g. production of new, biologically active compounds, increased resistance, altered appearance and productivity).

C. Method for Introduction of donor DNA.

[ ] electrical (specify) [ ] mechanical (specify)
[ ] biological vector if a biological vector is involved, describe:

- Construct and characterization (with references to published work and to proposal(s) covering preparation of the vector).
- History of prior use in genetic manipulation and recommended containment level.

D. Heredity of Introduced Traits.

- Reproductive capacity of experimental animals.
- Assessed heritability of transgene(s).
- Expected form of heredity of target phenotype(s).

Note: Address Item-17-18 only if other animals will be housed in the same facilities, but for other work; otherwise "Note applicable"

25. Considerations in the Design of Transgenic Animal Houses and in the Choice of Animal Containment Levels.

26. Concurrent Use of Transgenic Animal Houses.

A. Number and Composition of the Other Animals.
(number of animals of different species/strains).

B. Basic Nature of the Other Work.

- Use of the other animals, for example, in infectious disease work or in separate genetic manipulation work

C. Arrangements to keep transgenic animals separate from the other experimental animals (physical and temporal provisions).

D. Arrangements to identify and to account for individual animals.

27. End Use, Care and Disposal.

A. Intentions to breed if animals will be reared to full maturity (scheme for projected crosses).

B. End Use/Application of transgenic animals (only a brief description is necessary).

C. Arrangements for the care or disposal of transgenic animals at the conclusion of work.

28. Additional information, if any.

29. Signature (of Project Supervisor) and Date.

10.1. INSTRUCTIONS FOR THE PREPARATION OF THE PROJECT PROPOSAL FOR LABORATORY GENETIC MANIPULATION WORK

The Cover Sheet of the project proposal must be signed and dated by the project supervisor before submission to the IBC. For research work employing multiple project supervisors, the name and professional address of the supervisor preparing and submitting the proposal should be indicated under heading (1). and the said individual shall sign and date the proposal before submission to the IBC.

10.1.1 Names and Addresses

Names and addresses of institutions, laboratories, containment facilities and biosafety committees should be given in full. Ideally, telephone numbers, facsimile, e-mail etc. should be provided along with postal addresses. Foreign establishments must indicate both contacts in Pakistan and abroad.

Intentions to relocate must be promptly referred to the IBC so contact/links can be assured at all times.

10.2. PROJECT TITLE AND OBJECTIVES

Prominent intents should be indicated in the title or under the objectives.

Short and long-term objectives should be stated separately if the research work proposed is likely to continue for several years. Distinguish immediate from long-term goals. Provide a timetable of activities. It will permit the IBC to assess the proposal in sequence. As such, where the entire proposal does not merit endorsement (or where endorsement of later stages depends on the result of early work), the IBC may approve particular initial stages rather than having to reject the proposal as a whole, thus allowing preliminary work to begin and the proposal may be revised as results are compiled. Researchers should clearly indicate which parameters of the work requires preliminary endorsement.

10.3. MATERIALS AND METHODS

Give detailed description of the main experimental procedures. Details of special precautions/safeguards should be given, with reference to the specific risks and concerns identified in initial risk assessments.

10.3.1 Materials

For work with multiple donor DNA, hosts and/or vectors, indicate when and how each shall be used. As with providing a timetable of activities and distinguishing short and long term aims, clarifying these points will allow the IBC to assess the project in stages or modules.

Some details of the relevant history of prior work with components of the biological system should be provided, including track records of safety and biological containment. Indicate whether the DNA, hosts and vectors concerned are commonly or rarely subjected in

regulated work. Above all, identify any problem DNA, host, or vector with a history of unsafe use (e.g. hazards determined in initial risk assessments). If related host/vector systems have been field tested or released, a summary of the results/analysis would be appropriate.

10.3.2 Donor DNA

The origin of all donor DNA should be specified; scientific name and strain of the biological source(s), whether procured or constructed by the research team, and if procured, who made it. Researchers must account for how donor DNA was or will be constructed/cloned and should make clear as to whether several genes or species are involved. Some details of the biological source(s) are appropriate, for instance, whether a local or exotic strain and patterns of local distribution, particularly if imported.

10.3.3 Host Organisms or Tissues

The scientific name and strain of all host organisms and biological sources of host tissues need to be specified. In addition, researchers should briefly account for how the tissue culture will be prepared/grown. List all substantial hazards conveyed by host organisms or tissues, particularly, regarding pathogenicity and infectivity. Other details of the hosts may be appropriate, for instance, geographic distribution and biologically active compounds secreted.

10.3.4 Vectors

Work with biological vectors requires a concise description of the known vector properties (e.g. host range) in addition to nomenclature or identification. As with host organisms/tissues, list all substantial hazards borne, particularly, regarding pathogenicity and infectivity. For vectors which are genetically modified or constructed (e.g. retroviral vector), provide some details of the construct and methodology involved. A genetic map would also be appropriate.

10.3.5 Host/Vector Systems(s)

Researchers should elaborate, somewhat, on the predicted stability of introduced genetic traits (including, the localization and copy number of target genes, introduced gene expression, frequency of reversion to wild type characteristics) and the form of heredity of the target phenotype(s). Where applicable, also assess the likely stability of plasmids, phages, viruses, etc. in host organisms/tissues.

Identification of characteristics or markers should be detailed for references.

10.3.6 Auto-Ecology

Under 'Ecological Context' substantiate the level of biological containment provided by each host/vector system involved. Researchers need to briefly assess the viability of host/vector systems in the open environment (particularly, the natural tendency for invading wild populations, pests, or weeds). Also, they need to indicate any factors (including genetic modifications) which might limit growth, reproduction, and survival. Additionally, the IBC shall expect a review of the natural crossing possibilities to, or possibilities for exchange of genetic material with related

species/natural variants. Further details of the evolutionary potential should be presented under this heading.

10.3.7 Laboratories and Facilities

Clarify which phases of the work will be conducted in each of the laboratories and facilities identified, specify the certified containment level, and describe any special containment/safety features offered. Researchers must indicate whether permission has already been obtained for use, and if so, indicate the time period awarded and attach written confirmation.

10.4. DETAILS OF PERSONNEL

Attach a CV for each personnel involved in the proposed work, covering personal qualifications (e.g. education, training, professional history) and relevant research experience. Ideally, the general responsibilities of every individual should also be noted, so that the IBC may assess, on a case-by-case basis, whether personnel are adequately prepared and capable to handle the duties assigned.

It is essential that brief medical histories of all personnel at risk be included.

10.4.1. Commercial-in-Confidence

Researchers who wish to restrict information of commercial significance (e.g. trade secrets or confidential business reports) provided to the IBC and NBC in project proposal should mark the relevant material or portions "Commercial-In-Confidence".

IBC No.

National Bio-safety Committee (NBC)
Pakistan Environmental Protection Agency
Ministry of Climate Change &
Environmental Coordination,
Government of Pakistan
Islamabad.

IBC Assessment of Proposal for Laboratory Genetic Manipulation Work

1	**Name and full professional address of Principal Investigator submitting proposal.**	2	**Name(s) of other Principal Investigators responsible for the project. Please give their professional addresses if different from that in '1'.**
	Tel: **Fax:** **Email**		**Tel:** **Fax:** **Email:**

3	**Title of Project.**

4	**Intended date of commencement.**
	Expected date of completion.

5	**Assessment of the Project Proposal**			
5.1	Project Objectives & Methodology:	Approved [ ]	Not Approved [ ]	Inconclusive [ ]
5.2	Biological System:	Approved [ ]	Not Approved [ ]	Inconclusive [ ]
5.3	Site or Location:	Approved [ ]	Not Approved [ ]	Inconclusive [ ]
5.4	Safeguards and Contingency Plans:	Approved [ ]	Not Approved [ ]	Inconclusive [ ]
5.5	Timing and Period of Work:	Approved [ ]	Not Approved [ ]	Inconclusive [ ]
5.6	Details of Personnel:			
	• Experience and expertise	Approved [ ]	Not Approved [ ]	Inconclusive [ ]
	• Training and instruction	Approved [ ]	Not Approved [ ]	Inconclusive [ ]
	• Health	Approved [ ]	Not Approved [ ]	Inconclusive [ ]
	Other (please specify)	Approved [ ]	Not Approved [ ]	Inconclusive [ ]

6	**Assessment of supplementary information on Plant Work**

7	**Assessment of supplementary information on Animals**

8	**Risk category as assigned by the IBC**	Category 1 [ ]	Category 2 [ ]	Category 3 [ ]
9	**Laboratory Containment**	C1 [ ]	C2 [ ]	C3 [ ]
10	**Animal House Containment**	C1A [ ]	C2A [ ]	
11	**Plant Glasshouse Containment**	PH1 [ ]	PH2 [ ]	PH3 [ ]

SECTION B - IBC RECOMMDATION

12	**The Project has been reviewed by the IBC as assessed above and the Committee does not endorse [ ] the work has proposed; does not endorse [ ] the work with the following provisos.**

i) Provide additional information/documents on

ii) Follow conditions/amendments in your research as follows

.

13	The following special provisions must be adopted

i)

ii)

iii)

iv)

SECTION C - IBC REQUESTS TAC

14	The Project Proposal has been reviewed by the IBC and as assessed above, the Committee requires and requests MBC specific advice/action regarding the following

i)

ii)

iii)

iv)

15	**Signature of IBC Chairperson.**

Date / /

SECTION D – TAC REQUESTS NBC

16	The Project Proposal has been reviewed by the IBC and the MBC and as assessed above, the Committees requires and requests specific advice/action regarding the following

i)

ii)

iii)

iv)

17	**Signature of TAC Chairperson.**

Date / /

SECTION E

18	The NBC has reviewed the IBC/TAC assessment/request for advice and approved the following actions:

i)

ii)

iii)

iv)

19	**Signature of NBC Secretary.**

Date / /

10.5. INSTRUCTIONS FOR ASSESSMENT OF A PROPOSAL TO CARRY OUT LABORATORY GENETIC MANIPULATION WORK

The IBC Form for Assessment of a Proposal to carry out Genetic Manipulation Work serves, above all, to guide the Institutional Biosafety Committees in the consideration and evaluation of project proposal. These forms are meant to provide a framework for IBC, in assessing the experimental parameters of proposed research -- leading to the decision on whether to endorse the work at hand or propose amendments and provisos to be adopted as necessary. The IBCs must be clear in their evaluation of each component of the experimental system identified in the assessment form. Additionally, the committees should be thoughtful and thorough in drafting the various amendments and provisos to ensure an acceptable standard of biosafety for laboratory work under consideration. Regarding proposals for Category 3 work, special attention should be paid to determine which issues require NBC advice. Completed IBC assessments shall be submitted to the NBC through the TAC, together with corresponding project proposals, and the efforts of the committee will in turn, guide the NBC in reviewing the work proposed, as required.

The IBC shall assign a 10-digit number to all proposals; the first four digits is the institutional identification number; the second two digits refer to the serial number of theproposal received by the said institutional IBC during any calendar year and the last four digits refer to the month and the year of receipt of the proposal. (e.g. CAMB-01-0999, NIAB-05-1299, NARC-15-0100, etc.)

The IBC shall submit a completed assessment form to the TAC/NBC, attached to the corresponding project proposal, and retain a copy for records and reference. The TAC and NBC will use the IBC designated number but add "T" or "N" respectively to keep uniformity. Assessment forms must be signed and dated by the IBC Chairperson.

A clear and concise explanation is required for the IBC's position on each of the experimental parameters identified in the assessment form. The NBC shall expect some justification on IBC decisions to approve or not to approve the various components of the experimental system proposed. Where inconclusive, the IBC must indicate what information is lacking. As appropriate, references should be made to the relevant sections of the National Biosafety Guidelines in Genetic Engineering and Biotechnology.

Details of personnel need to be checked by the IBC, but the relevant attachments should not be forwarded to the NBC.

C H A P T E R 11

FORMAT OF PROJECT PROPOSAL FOR PERMISSION TO UNDERTAKE FIELD TRIALS

IBC No.

National Bio-safety Committee (NBC)
Pakistan Environmental Protection Agency
Ministry of Climate Change &
Environmental Coordination,
Government of Pakistan
Islamabad.

Cover Sheet

Proposal for Permission to Conduct Field Trials

1	**Reference numbers:** (IBC numbers for proposals, previously submitted from which this deliberate release proposal has developed.)

2	**Project title:**

3	**Name of organization:**

4	**Supervising IBC:**

5	**Project supervisor:**

Name:	*Position:*

Address:

Telephone:	*Fax:*	*Email*

6	**Location of release:**

7	**In which District/Province the release will take place?**

8	**When is the release to occur?**

9	**When is the release expected to end?**

10	Scale of release (number of animals involved, size of plot, etc.):

11	What is the size, scale, and timing of anticipated future releases?

12	Main objectives to be achieved from the release:

13	Signature of Principal Investigator submitting this proposal.
	Date / /

Section A - Introduction

14. Project Title

- Rationale of Studies

15. Project Objectives

- Overall Objective
- Specific Objectives

i)
ii)
iii)
.....…….....

- Objectives during 1st Year

- Time Schedule

Objective-1
Objective-2
Objective-3
….....

16. Intended Date of Commencement; .....

Expected Date of Completion. .....

17. Proposed Risk Category
[ ] Category 1 [ ] Category 2 [ ] Category 3

18. Anticipated Future Release and/or End Use.

Section B - Materials and Methods

19. Site of Field Work

- Location of trial and how plots are to be arranged on-site.
- Details of the physical environment and ecology.
- Facilities available on site.
- Reasons for the choice of location.

20. Scale of Field Work

- Approximate number of organisms involved and the size of test plots.

21. Methodology and Protocol

- Descriptions of the main experimental procedures.
- Indicate the developmental stages involved, and identify the control, test, andchallenge groups.

22. Precautions and Safeguards (please describe in full)

- Measures for containment of test plots and experimental organisms
- Arrangements for the disposal of experimental organisms, and for the clean-up of organic residues, at the completion of work
- Contingency plans

23. Results from Laboratory Tests of the Biological System

A. Characterization of Genetic Modification

- Stability of Introduced Genetic Traits
- Heredity of Genetic Inserts
- Level of expression and regulation of transgenes
- Traces of recombinant vectors in the final construct (where applicable)

B. Effects of Genetic Modification

- Changes in Phenotype and Novel Physiological Traits

C. Evolutionary Potential

- Competitive or Selective Advantage, conferred by genetic modification.
- Potential for Mutation and/or Adaptation to field conditions

D. Noxious or Harmful Characteristics

- Nature of the Harmful Agent
- Known and/or Likely Modes of Transmission

E. Ecological Context (Auto-Ecology)

- Viability in Open Environments
- Known predators and parasites
- Natural Crossing Possibilities to Related Species
- Propensity for Transfer of Genetic Inserts

24. History of Prior Field Work (with the experimental organism(s) or with related biological systems)

25. Assessed Course of Work

- Anticipated direct, and indirect ecological effects
- Possible secondary genetic effects

Section C - Personnel Involved with Research Work Proposed

26. Details of Personnel.

- Name, Qualification and Experience.
- Responsibilities and Duties.
- Medical History.

11.1. "CORE QUESTIONS" FOR CONSIDERATION IN THE PROPOSAL FOR GENERAL RELEASES

11.1.1. Species to be used

A. What is the species of organism to be used/released? Where relevant, give information on the strain, cultivar etc.

B. Is the parent organism or the GMO capable of causing disease or other ill-health in humans, plants, or animals? If so, what are the possible effects?

i) What is the origin of the inserted DNA?

ii) Does the inserted DNA come from an organism that causes disease or other ill-health in humans, plants, or animals? If so, what are the possible effects?

11.1.2. Aim

A. What is the intended use of the GMO?

B. What is the nature of the general release?

C. What is the nature of the activities that have the potential to lead to an unintended release?

11.1.3. Location

A. Location of the activities that have the potential to lead to an unintended release. For example, for genetically manipulated seeds to be imported for processing, the type of container, the location of entry of the seed into Pakistan, the transport routes, and the location of the processing facility.

B i) Relevant features of the physical environment in the area of the activities, particularly those which may minimize or exacerbate undesirable effects.

ii) Proximity of the site of activities to population centres, fields of agricultural activity, or the habitat of biota that might affect, or be affected by the proposed release?

11.1.4. Habitat and ecology

A. i) What is the natural habitat of the parent organism and its range?

ii) Where was the parent organism normally isolated?

iii) What is the distribution of the parent organism in the Indo-Pak sub-continent?

iv) Is the parent organism already present at or near the site of the activity? If so, provide available data on populations.

v) Is the parent organism exotic to the Indo-Pak subcontinent?

B. Are there known predators or parasites of the organism in the sub-continent? If so, describe.

C. Could release of the GMO prejudice any beneficial function of the parent organism in the environment?

11.1.5. Genetics of the GMO

A. What genetic modification has been made? (a detailed description of the steps undertaken in its construction).

B. Does the GMO have a potentially unstable genotype?

C. i) To what extent is the genetic modification characterized?
ii) What is the location of the inserted DNA in the final construct, and how many copies are present?
iii) What markers or sequences will enable the GMO to be identified in the laboratory and under field conditions.

D. i) What type of vector was used in the transfer? A description of the vector, showing the position of the inserted DNA and any other control sequences or markers in the vector, will be helpful to the reviewers.
ii) Can the vector transfer to other hosts? If so, provide information on its host range.
iii) Is the recombinant vector present in the final construct? If not, how was it removed?

E. If no vector was involved:

i) how was the DNA introduced?
ii) how many copies of the gene were inserted?

F. i) How does the modification change the phenotype of the organism to be used? Present data to demonstrate the effect of the modification, including level of expression and regulation of the genetic insert.
ii) What secondary genetic effects may be anticipated?

G. i) What intrinsic genetic features, if any, of the GMO regulate its survival in an unmanaged environment? How stable are these features?
ii) What genetic changes, if any, have been included in the GMO to limit or eliminate its capacity to reproduce or transfer its genes to other organisms?

11.1.6. Stability, survival and transfer

A. On the basis of contained experiments, provide data on:

i) the survival times of the GMO in habitats relevant to the activity;
ii) the growth rate (or generation time) of the parent organism and GMO in the ranges of environmental conditions characteristic for the place and date of the activity;
iii) the frequency of reversion or loss of the genetic change.

B. Is the GMO likely to be able to establish in the open environment outside the sites to be used for the proposed activities?

C. i) What is the capability of the GMO to disperse into the open environment?
ii) What are the dispersal mechanisms?

iii) Can the parent organism form long-term survival structures such as seeds or spores?

D. Is there any evidence that the inserted genetic trait can be transferred to other organisms found in the area and surrounding environment? If so,

i) to what organisms and at what frequencies? List the species that have been tested for transfer and explain the rationale for this choice.
ii) what transfer mechanisms are involved?
iii) what techniques have been used to demonstrate transfer?
iv) what are the possible adverse effects of the transfer?

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

F. Would you expect the GMO to show any competitive advantages over its unmodified parent in mixed populations under the conditions in the area to be used for the proposed activities? If so, what are the advantages?

11.1.7. Experimental procedures, monitoring and contingency plans

A. i) Detailed overall protocol for the activities that have potential to lead to an unintended release. Include procedures for transporting the GMO and accounting for transported organisms, if relevant.

ii) How many organisms will be involved in these activities?

iii) What will be the frequency of these activities (in terms of site years = number of sites X number of years of testing)?

iv) Will the activities be ongoing or for a limited period?

B. Details of any structures or procedures that will be in place to minimize the likelihood of establishment of the GMO in the environment.

C. If release of the GMO into the environment occurs, what might be the consequences (e.g. weediness of plants, problems related to feral animals, secondary ecological effects, toxicity of the GMO or its products to animals or humans)?

D. What contingency measures, if any will be adopted to rectify any unintended consequence if a hazard becomes evident during the course of the release?

E. Details of site supervision procedures and any safety procedures undertaken by staff.

11.1.8. Other assessments

A. Has a similar proposal for the construction of the GMO been assessed in any country in South Asia or South-East Asia, Far-East, Australia, Europe or American continent? Is so, please provide details, a copy of the proposal together with assessment report.

B. i) Have releases of the GMO been made before, either within or outside Pakistan? If so, what were the beneficial or adverse consequences? Provide or reports of previous assessments.

ii) Has an overseas country refused an application for the release or use of this organism?

iii) What factors might suggest greater or less risk with the proposed activity in Pakistan. Please provide references or reports of assessments

C. For an imported GMO, indicate the date of importation or intended importation, documentation of clearance or assessment from the National Regulatory Body of the country of export.

D. Is there any reason to think that the GMO, if released, could constitute a hazard, not discussed elsewhere in the proposal, a) in the area designated, or b) elsewherein Pakistan?

E. If the organisms are to be consumed as food, please attend to the following additional points.

i) Is the parent organism or the donor organism already used in food production or eaten as food? If so, i) at what level of daily/weekly intake, and ii) is any processing needed or commonly used before consumption?

ii) - Does the GMO produce metabolites which may have adverse effects on the consumer (humans or animals)? If so elaborate. Provide available date on toxicology, allergenicity and other possible adverse effects.

- Can any product of the GMO concentrate in the food chain to levels which may become toxic? If so, elaborate.

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

iv) Will the GMO be processed during the production of the food? If so, elaborate.

v) Will the GMO be the major component of the food, or is it in small numbers in the final product (e.g. yeast cells in bread making)?

F. Any other information that could assist assessment of the proposal

11.2. ADDITIONAL POINTS IF YOUR PROPOSAL IS FOR RELEASE OF MICROORGANISMS

11.2.1. Aim

A. i) What is the intended eventual use of the GMO?

ii) What is the aim of the trial?

11.2.2. Location

A. Describe the size of the release, and, where relevant, the area of land to be used, and its location. Provide location and site maps where relevant.

B. i) What are the reasons for the choice of location?
ii) Describe in detail the relevant features of the physical environment, particularly those which may minimize or exacerbate undesirable effects.
iii) How close is the site to population centres, FIELDS of agricultural activity, or the habitat of biota that might affect, or be affected by the release?

11.2.3. Experimental procedures, monitoring and contingency planning

A. i) Describe in detail the overall experimental protocol for the release, including the protocol for control, test, and challenge organisms, if appropriate.
ii) How many organisms are to be released?
iii) How many releases of the GMO are proposed?

B. i) What are the arrangements for producing the GMO in quantity,transporting it to the site, and accounting for the transported organisms?
ii) How will the GMO be released?

C. i) What methods are to be used to test for batch to batch consistency if large scale production is required to produce GMOs for release?

ii) What specific measures have been taken or will be taken in the production process to ensure the quality/purity of the GMO to be released?

D. i) How will the survival of the GMO be monitored? Provide a description of techniques for monitoring the presence of GMOs or transferred genetic material beyond the primary site, including specificity, sensitivity and reliability of detection methods.
ii) If the release is likely to affect the characteristics or abundance of other species, how will this be monitored?
iii) How will transfer of the introduced gene to other species be monitored?

E. i) What potential hazards or deleterious effects can be postulated.
ii) Measures that will adopted to reduce dissemination of the GMO.
iii) If transfer of the inserted genetic trait to other organisms could result in adverse consequences, what methods will be used to minimize these effects?

F. i) Will the GMO remain in the environment after the release? If so, a) for what period of time, and b) what might be the consequences?
ii) Will measures be taken to reduce populations or dispose of the GMO once the release is completed? If so, provide details.
iii) What monitoring is to be undertaken after the release is completed?
iv) Provide details of the procedures to be used for collection, storage and transport of seeds collected from the GMO, if appropriate.

G. What contingency measures will be adopted to remove the GMOs if a hazard becomes evident during the course of the release?

i) What will be the site supervision procedures and any safety procedures undertaken by staff? What is the distance of the site from the location of the IBC.

ii) What measure have been adopted to train the staff responsible for conducting the release.

11.3. ADDITIONAL POINTS IF YOUR PROPOSAL IS FOR THE RELEASE OF PLANTS

A. Has the parent plant an extended history of cultivation and of safe use? If not, explain.

B. 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 GMO (e.g. reduced fertility, increased prevalence, production losses, grain shedding)? Indicate the likelihood of these events.

C. i) Describe the mechanism of pollen spread (by insect vectors or by other means) in the plant.

ii) Provide available data on pollen viability for the plant.

iii) Indicate potential pollinators and their range and distribution in Pakistan.

D. i) Are any members of the genus of unmodified parent known to be weeds inany environment? If so, specify.

ii) Are there literature reports on cross-pollination between the species to which the GMO belongs and wild relatives known to be weeds? If so, please list.

E. i) Provide quantitative data on successful cross-pollination between the plantand its wild relatives.

ii) If you know that sexually compatible plants live near the site of the release, give details and quantify the chances for cross-pollination.

iii) If cross-pollination occurred, would the resulting plants survive/compete well? If not, why not?

F. If there is any possibility that the imparted characteristic could be integrated into other species:

i) Would it have the potential to effect the distribution and abundance of the other species? If so, specify. Data on the factors which normally control populations of these other species in the natural environment (e.g. pathogens, herbivory, physiological stress) may be relevant;

ii) Could it have any other adverse consequence?

iii) Has any attempt been made to minimize the risk (e.g. by imparting male sterility or other means of reproductive isolation)? If not, why not?

G. i) Will the plants in this release set seeds? If not, is this planned for later releases?
   ii) If plants are allowed to set seed, does the mature seed normally remain contained within an year, capsule, pod etc. so that practically all of the seed can readily by harvested, or is the seed shed soon after it matures?
   iii) Can the seed be dispersed by natural mechanisms? If so, describe.
   iv) Are the seeds capable of being dormant for a long time? If so, how long?

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

I. How might the plan's competitive advantage (fitness) be changed (i) in the agricultural setting; (ii) in the natural environment? Explain.

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

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

L. What secondary ecological effects might result from release of the GMO (e.g. effect on endangered native species, resistance of insect populations to an insecticide, reduction or increases in numbers of prey or parasites)?

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

   i) Provide data on the degradability, selectivity and toxicity of the chemical concerned;
   ii) What is the agronomic significance of the chemical?
   iii) What is the biological activity of the chemical?
   iv) How is the chemical applied and used?

N. If the construct involves resistance to a herbicide, explain or describe:

   i) The farming system into which the crop is to be integrated and the effect the release will have on this system;
   ii) What impact the release will have on use of the herbicide to which the GMO has been made resistant (provide forecasts on areas to be sprayed and volumes to be applied).
   iii) What impact the release will have on total use of other herbicides and insecticides;
   iv) What impact the release will have on weed control.
   v) How the release will affect programs designed to involve environmentally friendly chemicals or practices;

vi) The development and operation of future Integrated Pest Management (IPM) strategies for farming system in which the GMO is used. In particular, describe how the strategies would minimize or avoid (a) the potential for the GMO itself to become an environmental weed; (b) the potential emergence of herbicide-resistant weeds; and (c) the potential persistence of the transgene or GMO in the farming system such that herbicide-resistant volunteer plants appear within subsequent crops of a rotational system.

11.4. ADDITIONAL POINTS IF YOUR PROPOSAL DEALS WITH MICRO-ORGANISMS LIVING IN OR ON ANIMALS

A. What is the animal host species?

B. Has the parent organism an extended history of use in agriculture? If so, please elaborate.

C. i) What new capacity will the GMO provide for the host species? (e.g. ability to degrade plant or pasture toxins)?
ii) What secondary effects can be postulated from conferring that capacity on the host?

D. Will the competitive advantage or ecological fitness of the host be altered? Provide data to support your answer.

E. Is there any evidence that the GMO might be capable of establishing in or on other animals, including feral animals? If so, what are these animals and what arethe effects?

F. What other effects (including secondary effects) are likely on other plants or animals in the agricultural and natural environments?

G. What secondary effects can be postulated from the introduction of the GMO 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)?

H. Will the GMO be excreted or otherwise leave the animal? If so, for how long does it survive outside the animal?

i) What is survival and dispersal of the GMO in natural waters and soil?
ii) What could be the effects of the GMO on water quality?
ii) Does the GMO produce spores?
iv) Is the GMO resistant to desiccation?

I. What sterilizing and anti-microbial agents are active against the GMO? Is the GMO susceptible to UV and ionizing radiation?

11.5. ADDITIONAL POINTS IF YOUR PROPOSAL DEALS WITH MICRO-ORGANISMS AS LIVE VACCINES

A. i) What disease is to be controlled by the use of this vaccine?
ii) On what host species is the vaccine to be used?
iii) What is the host range of the parent organism from which the vaccine was constructed?

B. 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 (District/Province), if applicable.

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

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

E. 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 (e.g. immunosuppression or superimposition of other disease) or by other treatments (e.g. drugs)? If so, elaborate.

F. 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?

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

H. i) If the Proposal is for permission for field trials, is it proposed to dispose off waste which contains vaccine organisms? If so, describe the arrangements.
ii) What is the fate of the vaccinated animals at the conclusion of the trial?

I. Will the vaccinated humans or animals carry live vaccine organisms at the end of the trail? If so:

i) Will they be likely to disseminate the live vaccine organisms to their family contacts or to the general population?
ii) What measures, if any, will be taken to minimize this possibility?
iii) Will the organisms be able to cross the placenta?

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

K. Is the vaccine likely to have any deleterious effects on pregnant humans or animals? If so, specify. For humans provide data from animal models.

L. Is the vaccine teratogenic (i.e. causing developmental defects) for the foetus at any stage of gestation? If so, elaborate.

M. Does the GMO produce spores?

N. Is the GMO resistant to desiccation?

O. What sterilizing and anti-microbial agents are active against the GMO? Is the GMO susceptible to UV and ionizing radiation?

11.6. ADDITIONAL POINTS IF YOUR PROPOSAL DEALS WITH MICRO-ORGANISMS NOT FALLING UNDER SECTIONS 11.4 & 11.5

(Which are associated with plants and microorganisms which might be applied to modify the physical or chemical environment (e.g. microorganisms to modify soil properties).

A. For microorganisms associated with plants:

i) What is the partner species of plant? Describe the specificity of the interaction and indicate the range of plant species with which the GMO can interact.
ii) Has the parent organism an extended history of use in agriculture? If so, please elaborate.
iii) What is the effect of the GMO on the partner plant species and how will this be monitored?
iv) What other secondary effects might the GMO have on the plant?
v) Does the modification cause any change to the range of host plant species available to the organism?
vi) What effect of the GMO, if any, is anticipated on the distribution and abundance of the host plant species and other species with which the organism can interact.

B. If the plant species are food crops will, it affect the suitability of the resultant produce for human or animal consumption? If so, explain.

C. What are the effects expected on soil chemistry (e.g. pH, mineral leaching, chelation, nutrient levels)?

D. What is the survival and dispersal of the GMO in natural waters and soil?

E. What could be the effects of the GMO on water quality?

F. Does the GMO produce spores?

G. What effects might the GMO have on soil organisms which are known to be beneficial to plants (e.g. Rhizobium, Azospirillum, Frankia and mycorrhizal fungi) and are likely to be in the test area?

H. What is known about interactions between the GMO closely related microorganisms in the partner plant (if applicable) or the environment of the release site?

I. For GMOs associated with plants, what effect might the GMO have on insects, birds and animals (including humans) which may eat the plant?

J. Does the GMO exchange genetic material with known plant pathogens? If so, elaborate.

K. Is the GMO resistant to desiccation?

L. What sterilizing and anti-microbial agents are active against the GMO? Is the GMO susceptible to UV and ionizing radiation?

11.7. ADDITIONAL POINTS IF YOUR PROPOSAL DEALS WITH ORGANISMS FOR BIOREMEDIATION

A. i) What is the target substrate for bioremediation?
ii) What effect does the parent organism have on the target substrate?
iii) What effect does the GMO have on the target substrate?

B. What other substances can be metabolized by the GMO which cannot be metabolized by the parent organism?

C. Will the GMO be self-sufficient if added to the contaminated site or will additional measures be required (e.g. provision of supplementary nutrients and growth factors or other environmental modifications)?

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

E. What effects might the GMO have on water, air or soil quality?

F. What effects might the GMO have on organisms which ingest it?

G. Will the GMO be dispersed from the site of application? If so, describe the mechanisms involved and the consequences.

11.8. ADDITIONAL POINTS IF YOUR PROPOSAL DEALS WITH ORGANISMS FOR BIOLOGICAL CONTROL

A. i) What is species targeted for biological control?
ii) What direct effects does the parent organism have on the target species?
iii) What direct effects does the GMO have on the target species?

A. i) What is the host range of the GMO? If the host range of the GMO is likely to be different from that of the parent organism, explain why.
ii) What non-target organisms have been tested for susceptibility to the GMO?
iii) What is the rationale for the choice of species tested?

B. How is the GMO transferred from one target individual to another and what factors affect this transferability?

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

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

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

H. If the modified genetic traits can be transmitted to other organisms which are likely to be in the environment (see A19), are these other organisms likely to affect non-target species?

11.9. ADDITIONAL POINTS IF YOUR PROPOSAL DEALS WITH ORGANISMS TO BE CONSUMED AS FOOD

11.9.1. If the product is enzyme:

A. Is the parent organism or the donor organism already used in food production or eaten as food? If so, (i) at what level of daily/weekly intake, and (ii) is any processing needed or commonly used before consumption?

B. i) Does the GMO produce metabolites which may have adverse effects on humans or animals? If so, elaborate. Provide available data on toxicology, allergenicity and other possible adverse effects.
ii) Can any products of the GMO concentrate in the food chain to levels which may become toxic? If so, elaborate.

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

D. Is the GMO to be processed during the production of the food? If so, elaborate.

E. Is the GMO the major component of the food as eaten, or is it in small numbers in the final product?

11.9.2 If the product is fish and aquatic organisms such as crustaceans

A. i) Could the GMO produce any 'new' metabolites or toxins likely to have deleterious effects on parasites or predators? If so, elaborate.
ii) What other unintended effects may result from the release? Your answer should include consideration of the effect of the GMO on the community ecology at the release site.
iii) Are any of the likely gains directly linked to losses in other characteristics of the organisms?

B. i) Will the GMOs in this release be allowed to breed? If not, is breeding planned for later releases or commercial use?
ii) Are the arrangements for handling any offspring the same as those for the experimental organisms? If not, please specify the arrangements.

C. 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, an elaborate its effects.

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

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

F. Has any experimental work been done on phenotypic expression of the novel genetic material in naturally occurring organisms (e.g. cross-breeding of GMOs with wild or domesticated animals)? If so, what were the results?

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

H. 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.

I. What mechanisms will be used to prevent dispersal of the GMO into other ecosystems?

11.9.3 If the product is Invertebrates

A. i) What effects might the GMO have on the food chain?

ii) Could the GMO produce any 'new' metabolites or toxins likely to have deleterious effects on parasites or predators? If so, elaborate.

iii) What other unintended effects may result from the release? Your answer should include consideration of the effect of the GMO on the community ecology at the release site.

B. Will the GMOs in this release be fertile? If not, is it intended to use fertile organisms in later releases?

C. Do populations of the parental organism exit in Pakistan? If so, do these populations cause agricultural, environmental or public health problems or benefits? Specify the problems or benefits.

D. i) 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.

ii) What is the likelihood of the novel genetic material entering gene pools of natural populations?

iii) Can the changed or added genetic material be transmitted to any other species? If so, specify the mechanism of transfer and list the species.

E. Has any experimental work been done on the phenotypic expression of the novel genetic material in other genetic backgrounds (e.g. cross-breeding of modified strains with wild animals)? If so, what were the results?

F. 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?

E. What mechanisms will be used to prevent dispersal of the GMO into other ecosystem?

11.10. INSTRUCTION FOR THE PREPARATION OF THE PROJECT PROPOSAL FOR PERMISSION TO UNDERTAKE FIELD TRIAL WORK

The Project proposal for Permission to undertake Field Trial Work (alongwith all attachments and supplements) will serve as the principal source of reference for the IBC in the consideration and approbation of field work regulated under these Guidelines. On the basis of information provided in, and of risks/concerns that may be inferred from these proposals, the IBC shall classify field work and determine additional biosafety measures to be adopted/implemented as necessary, including site relocation and procedural amendments. Proposals may also be reviewed by the TAC the NBC, and whatever details provided will constitute the framework for assessment and recommendations.

Recognizing that assessments depend on the written proposals, researchers should be thorough yet concise, and clear as to their intentions, so that the committees may readily and fully understand the nature of proposed work. All important details should be included. Prominent intents should be stressed. All data and relevant scientific literature must support your statements. Full disclosure of data or published literature, that allude to potential adverse effects of Genetically Modified Organisms or products thereof to be released, must be made in the project proposal/application. It is critically important that all relevant core questions on Pages 67 to 70 are seriously considered and included in the proposal/application under appropriate "headings" or as an attachment. References should be fully documented, and attached to the proposal/application. In making an assessment, the IBC shall consider together with other relevant matters, whether data obtained in the laboratory or under contained conditions provide sufficient basis to allow release of the GMO and/or products thereof. The IBC shall consult and discuss with the proponent, make suggestions for revision of the proposal or require further experimental work under contained conditions as it deems necessary.

The project supervisor must submit three typed, completed project proposals to the supervising IBC (which shall forward two copies to the TAC /NBC for information/processing) and retain one copy for records and reference. For work supported by two or more institutions, all IBCs must be notified.

Cover Sheet of the project proposal must be signed and dated by the project supervisor before submission to the IBC. For field work employing multiple project supervisors, the name and professional address of the supervisor preparing and submitting the proposal should be indicated under heading (1) and the said individual shall sign and date the proposal before submission to the IBC.

11.11. IMPORTANT DIRECTIVE

Researchers must procure a copy of the corresponding Project Proposal for Assessment of Laboratory Genetic Manipulation Work, which precedes the initial genetic engineering of this biological system to be field tested. Attach this form to the back page of the Project Proposal for Assessment of Genetic Manipulation Field Work before submission to the responsible IBC. Some information in this proposal is critical to IBC assessment.

11.12. COMMERCIAL-IN-CONFIDENCE

Researchers who wish to restrict access to information of commercial significance (e.g. trade secrets or confidential business reports) provided to the IBC and NBC in project proposals, should mark the relevant material or portions "Commercial-In-Confidence".

11.13. SUBMISSION OF PROPOSALS

A proposal must be submitted to the IBC responsible for the laboratories which intends to carry out field trials before work commences for all work involving genetically manipulated microbes, plants or animals. Where more than one organization is involved, all IBCs must be informed and reported in the proposal. The project supervisor will submit three completed project proposals to the supervising IBC which shall forward two copies to the TAC/NBC for information/guidance/approval and retain one copy for records and reference.

The IBC will assign each proposal a 10 digit number; check the information provided regarding the proposed biological system, the physical facilities to be used and the details of the members of the project team. It will then make its assessment of the proposal with regard to the proposed level of physical containment, and the adequacy of the experience of the members of the team for carrying out the proposed work.

11.14. APPROVAL OF PROPOSALS AND COMMENCEMENT OF WORK

Work assessed as low to medium risk may commence after a proposal has been assessed and approved by the IBC. Work must be conducted as recommended by the IBC. Work assessed as high risk by the IBC must not commence without the specific approval of the NBCas the case may be.

11.15. CONDUCT OF WORK

The Principal Investigator must ensure that the recommendations of the IBC, and NBC are complied with, and that the physical containment requirements and procedures are met during the course of the work.

11.16. REPORT ON FIELD TRIAL

When the field trials have been completed, a report must be prepared three copies submitted to respect IBC. The IBC will retain and forward two copies to NBC for record.

IBC No.

National Bio-safety Committee (NBC)
Pakistan Environmental Protection Agency
Ministry of Climate Change &
Environmental Coordination
Government of Pakistan
Islamabad.

IBC Assessment of Proposal for Field Trial

1	**Reference numbers:** (IBC numbers for proposals, previously submitted from which this deliberate release proposal has developed.)

2	**Project title:**

3	**Name of organization:**

4	**Supervising IBC:**

5	**Project supervisor:**	
Name:	*Position:*	
Address:		
Telephone:	*Fax:*	*Email*

6	**Location of release:**

7	**In which District/Province the release take place?**

8	**When is the release to occur?**

9	**When is the release expected to end?**

10	Scale of release (number of animals involved, size of plot, etc.):

11	What is the size, scale and timing of anticipated future releases?

12	**IBC assessment:** (Give an evaluation of the project including a comment on the project supervisor's capability to manage the work, the adequacy of the project design, site selection and contingency plans.)

SECTION B - IBC RECOMMDATION

13	**The Project has been reviewed by the IBC as assessed above and the Committee does not endorses [ ] the work has proposed; endorses [ ] the work with the following provisos.**
i) Provide additional information/documents on ii) Follow conditions/amendments in your research as follows .	

14	The following special provisions must also be adopted
i) ii) ii) iv)	

SECTION C - IBC REQUESTS TAC

15	The Project Proposal has been reviewed by the IBC and as assessed above, the Committee requires and requests MBC for specific advice/action regarding the following

i)

ii)

ii)

iv)

16	**Signature of IBC Chairperson.**

Date / /

SECTION D – TAC REQUESTS NBC

17	The Project Proposal has been reviewed by the IBC and the MBC and as assessed above, the Committees requires and requests specific advice/action regarding the following

i)

ii)

ii)

iv)

18	**Signature of MBC Chairperson.**

Date / /

SECTION E

19	The NBC has reviewed the IBC/MBC assessment/request for advice and approved the following actions:

i)

ii)

ii)

iv)

20	**Signature of NBC Secretary.**

Date / /

11.17. INSTRUCTIONS FOR ASSESSMENT OF A PROPOSAL TO CARRY OUT FIELD TRIAL

The IBC Form for Assessment of a Proposal to carry out Field Trials of Genetically Manipulated CMO's serves, above all, to guide the Institutional Biosafety Committees in the consideration and evaluation of project proposal. These forms are meant to provide a framework for IBC, in assessing the experimental parameters of proposed work -- leading to the decision on whether to endorse the proposal or propose amendments and provisos to be adopted as necessary. The IBCs must be clear in their evaluation of each component of the experimental system identified in the assessment form. Additionally, the committees should be thoughtful andthorough in drafting the various amendments and provisos to ensure an acceptable standard of biosafety for field work under consideration. Special attention should be paid to determine which issues require direct TAC/NBC endorsement. Completed IBC assessments shall be submitted to the TAC/NBC, together with corresponding project proposals to assist them in reviewing the work proposed, as required.

The IBC shall assign a 10 digit number to all proposals; the first four digits is the institutional identification number, the second two digits refer to the serial number of theproposal received by the said institutional IBC and the last four digits refer to the month and the year of receipt of the proposal. (e.g. CAMB-01-0999, NIAB-05-1299, NARC-15-0100, etc).

The IBC must submit a typed, completed assessment form to the NBC, attached to the corresponding project proposal, and retain a copy for records and reference. The TAC and NBC will use the IBC designated number but add "T" or "N" respectively to keep uniformity. Assessment forms must be signed and dated by the IBC Chairperson to be received by the TAC or NBC. Where appropriate, IBC advice and copies of the completed assessment form should besent to those regulatory agencies duly constituted to manage the planned release of Genetically Modified Organisms, or with the legal responsibility to approve the end use of such organisms.

A clear and concise explanation is required for the IBC's position on each of the experimental parameters identified in the assessment form. The TAC/NBC shall expect some justification on IBC decisions to approve or not to approve of the various components of the experimental system proposed. Where inconclusive; the IBC must indicate what information is lacking. As appropriate, reference should be made to the relevant sections of the NBC Biosafety Guidelines in Genetic Engineering and Biotechnology for Field Work and Planned Release, 2005.

Details of personnel need to be checked by the IBC but the relevant attachments should not be forwarded to the NBC.

Some Specific Provisions

The IBCs may authorize or commission research work immediately, upon endorsement of the project proposals. Measures for the control and containment of field work shall observe the rudimentary standards, in current or past practice, as appropriate to the particular organism under investigation.

IBC assessments should be attached to the top sheet of the corresponding project proposals and submitted to the TAC/NBC at the earliest possible. The NBC shall assume direct responsibility for endorsing such proposals, and for preparing any terms of approval, additional to IBC recommendations. Measures for the control and containment of field work must comply with NBC and IBC advice/instruction and with the relevant criteria presented in Chapter-4 of the Biosafety Guidelines in Genetic Engineering and Biotechnology.

C H A P T E R 12

FORMAT FOR APPLICATION FOR COMMERCIALIZATION OF GENETICALLY ENGINEERED ORGANISMS AND PRODUCTS THEREOF

When GMOs and/or products thereof have been adequately field tested and enough data accumulated to show that the experimental organisms and its products are free from any risk, an application may be made requesting that the said GMO or its product should no longer be considered as regulated material. The application/project proposal must contain, sufficient detail, field performance data, as well as relevant molecular biology data to ensure that the reader would be able to ascertain that the material poses no risk. The proposal/application may also include views/decision on similar product (not the same product) and data obtained elsewhere (not necessarily by the applicant) whether favourable or unfavourable to the positionof "no risk status".

The succeeding pages present a suggested format for preparing an application.

IBC No.

National Bio-safety Committee (NBC)
Pakistan Environmental Protection Agency
Ministry of Climate Change &
Environmental Coordination
Government of Pakistan
Islamabad.

Cover Sheet

Application for Commercialization of Genetically Engineered Organisms and Products Thereof

1	Name and full professional address of Principal Investigator submitting proposal.	2	Name(s) of other Principal Investigators responsible for the project. Please give their professional addresses if different from that in '1'.
Tel: **Fax:** **Email**		**Tel:** **Fax:** **Email:**	

3	Name of Products

4	Summary of Field Data:

a) Duration of Field Test:_____ b) Sites of Field Test: _____

c) Scale of Field Tests:

d) Description of Field Tests:

e) Results of Field Tests:

f) Conclusion of the field results:

5	Certificate:

The undersigned certifies that, to the best of his/her knowledge and belief, this application includes all data, information, and views relevant to the matter, whether favourable or unfavourable to the position of the undersigned, which is the subject of the application

Signature of the Applicant:

6	Signature of Principal Investigator submitting this proposal.
	Date / /

7. Rationale for Development of the Organism/Product

8. The Organism or its Product

   a) Unmodified organism (e.g. cotton/rice as a crop)
   b) Taxonomy of the organism
   c) Genetics of the organism
   d) Mode of reproduction
   e) Characteristics of the unmodified organism/product

   If the organism is a crop plant, please proceed to (f) and (g).

   f) Modes of gene escape in the environment
   g) Weediness (the organism is a crop or plant)

9. Description of the Modification/Transformation

10. Donor Genes and Regulatory Sequences

    a) Gene of interest
    b) Marker gene

11. Genetic Analysis of Field Performance

    a) Southern Gel Analysis
    b) Expression of inserted genes
    c) Characteristics of the modification/improvement
    d) Characteristics of the specialized feature introduced as a modification.
    e) Mendelian inheritance
    f) Mycotoxins (in the case of plants)

12. Environmental Consequences of the Modification

    a) Specific advantages
    b) Current uses of unmodified organism/product, normal practices, and disadvantages
    c) Proposed use of the modified organism/product with economic and social advantages
    d) Possible side-effects
    e) Effects on related organisms
    f) Vertical transfer of the new genes
    g) Horizontal transfer the new genes

13. Adverse Consequences of the Modification

14. References

12.1. INSTRUCTION FOR THE PREPARATION OF THE APPLICATION FOR COMMERCIALIZATION OF GENETICALLY ENGINEERED ORGANISMS AND PRODUCTS THEREOF

12.1.1 Description of the biology of the non-modified recipient organism should include taxonomy, genetics. If the organism is plant, then pollination, and evidence of reported weediness (e.g., noting whether the crop or sexually compatible species is listed in the relevant publications of the Weed Society of America), and discussion of sexual compatibility with wild and weedy free-living relatives in natural crosses or crosses with human intervention should be included. The applicant should provide the source of recipient (cultivar name or accession number) and the weed status of its sexually compatible relatives.

12.1.2 The applicant should explicitly identify the lines to be considered in the application and the cultivars from which they are derived. If there are multiple lines, each line must be given a unique identifier and listed in the application. For virus-resistant plants, applicants should provide in an additional section on the nature of the virus that provided the sequences encoding the resistance phenotype:

i) The taxonomic name of the virus including family, genus, and strain designation including any synonyms;
ii) The type of nucleic acid contained in the virus;
iii) Whether the infection is systemic or tissue specific;
iv) Whether the virus is associated with any satellite or helper viruses;
v) The natural host range of the virus;
vi) How the virus is transmitted;
vii) If transmitted by a vector, the identity of the vector including mode of transmission (e.g., persistent or nonpersistent);
viii) Whether any synergistic or transcapsidation interactions with other viruses under field situations have been reported in the literature, and
ix) The location and the name of the host from which the plant the virus was originally isolated.

12.1.3 The above information can be provided in a table format. This information can be supplemented by listing references that report the host range, insect vectors, etc., for the virus.

For *Agrobacterium*-based transformation protocol, the applicant must indicate how Ti plasmid-based vector was disarmed (i.e., all tumorigenic DNA was removed). Applicants can provide citations that describe the transformation procedure. However, any significant modifications of transformation, strain designation, etc. should be described.

For other methods of transformation, the applicant can describe the sources of various components of the plasmid (or other DNA including possible carrier DNA) and method of transformation by citation. However, any significant modifications in transformation, tissue regeneration, etc. should be described.

12.1.4 The applicant must provide a detailed restriction map of the plasmid that is sufficient to be used in the analysis of Southern data. Description of added restriction sites is helpful in interpretation of Southern data and should be provided.

12.1.5 In general, it is always prudent to analyze data statistically when such analysis is possible. When unpublished information or an opinion has been supplied by a scientific expert,a letter communicating the information should be included in the application. If the unpublished information provided is data resulting from scientific research, then these data can be provided as a personal communication either in a letter from the researcher or in the text of the project/application. In either case the materials and methods, data analysis, and discussion of the data analysis should be provided in detail. Unsupported assertions about the results of the experiment are not acceptable.

Applicants must report any differences noted between transgenic and non-transgenic plants that are not directly attributed to the expected phenotype. Differences observed could include changes in morphology, rates, other changes in overwintering capabilities, insect susceptibilities, diseases resistance, yield [if plants, pollen viability, seed germination and agronomic performance etc.]. Applicants must also note the types of characteristics that were compared between unmodified and modified organism.

The applicant should describe whether data submitted are from inbred or hybrid plants; if hybrid plants, state which generation.

If the organism is Plant, please address the following:

12.1.6 State whether data with respect to plant performance were generated in a greenhouse or field environment. If from the field, indicate how many sites, states, and number of years the data represents. Seed germination, seed dormancy, seed production, growth rate, and other data relating to the plant's performance will be required when the nature of the gene and the biology of the plant (including sexually compatible relatives) warrant such data. This type of data will usually not be required for plants that are highly domesticated (e.g., corn), exclusively self- pollinating (e.g., soybean), and male sterile. Also, for the plants that have high seed germination rates (>90%), and whose phenotypes are unlikely to affect performance with respect to weediness or fitness (e.g., delayed ripening or oil seed modification). Phenotypes that might require performance data (depending on the plant) include the following but are not limited to these attributes: cold tolerance, salt tolerance and tolerance or resistance to other biotic or abiotic stresses.

12.1.7 Southern analysis should include DNA isolated from non-modified recipient, all or selected transformed lines, and the vector. Parental plasmid DNA (e.g., PUC 18) not containing intended donor genes may be labeled and hybridized to Southern blots to demonstrate that only the intended sequences have been incorporated in the genome of the transgenic plant. Restriction enzymes to be used might include enzymes that do not cut within the transforming plasmid but will cut the entire insert into one fragment from the DNA of the transgenic plant.

In the case of an *Agrobacterium*-based transformation system, the applicant should determine if genes that reside outside the LB/RB are inserted in the genome of the regulated cultivar. If a complete copy of any of these genes is present, the applicant should determine

whether it is expressed in the plant. For direct transformation systems, applicants should determine which sequences are inserted in transgenic plants and whether they are expressed. PCR analysis may be used to prove that only the targeted DNA has been incorporated. Sequencing of the transgene in plant and adjacent sequences is not required. Determination of the number of copies of integrated transgenes is not required, but the number of insertions may be used to support analysis of inheritance data.

12.1.8 If the inserted DNA sequence order is complex, as is often the case for plants engineered via direct transformation systems (e.g. electroporation, polyethylene glycol transformation of protoplasts, or particle bombardment techniques), the applicants should summarize the data by providing the following information for all the genes (whether under the direction of plant or bacterial promoters), is there a complete copy of the gene present in regulated article? Is the protein expressed in the plant? If multiple complete copies of a gene are present, applicants do not have to determine if each copy of the gene is expressed. It is very helpful to provide a table, that summarizes the results and indicates where specific data is to be found.

12.1.9 Mendelian inheritance data and Chi square analysis for at least 2 generations are appropriate to demonstrate whether the transgene is stable inserted and inherited in Mendelian fashion. Such data are generally not necessary for vegetatively propagated infertile crops such as male-sterile potatoes.

12.1.10 RNA-Northern analysis is generally not required except for virus-resistant plants. However, such analysis may be necessary for ribozyme, truncated sense, or antisense constructs, when protein levels cannot be provided.

12.1.11 Proteins-Expression levels of gene(s) of interest and marker genes in various tissues, developmental stages of plant, and experimental conditions (induced or non-induced) are required. Assays can be of enzyme activity. Serology, ELISA, and Western blots may also be used. Describing the source of the immunogen is critical for serological analysis.

For virus resistant plants, the amount of viral transgene RNA produced should be determined and compared to the amount of the RNA produced by the viral gene in an infected non-transgenic plant. Applicants should address whether the transgenic RNA (or protein) is present in the same tissues as are infected during natural infections. In addition, provide the amount of both coat proteins (i.e., from the transgene and the naturally infecting virus) produced in the transgenic plant singly infected with the widely prevalent viruses in the U.S. that normally infect the recipient plant (contact NBC for the list of these viruses). For comparison, provide the amount of both coat proteins produced in the non-engineered plant in mixed infections of the virus from which the coat protein gene was derived and the same widely prevalent viruses used in the single infection study. Provide description of symptoms of infected plants in all cases.

12.1.12 For all diseases and pathogens surveyed, names of the diseases and the scientific names of the pathogens should be provided. Data from field tests in foreign countries are acceptable. If the data on diseases and pests were obtained in the foreign country, the applicant should submit information about the distribution of those pests; disease or pathogens in Pakistan or the sub-continent. Disease and pathogen susceptibility on wild type and transgenic plants should be determined preferably from natural infestations. However, if applicant must use direct

inoculations: i.e., with virus resistant transgenic plant, the source and taxonomic classification of the virus should be provided.

12.1.13 Certain plants have minute quantities of known toxicants which may adversely impact non-target organisms and beneficial insects: e.g., tomatine in tomatoes, cucurbitin in cucurbits, gossypol in cotton etc.. If such plants are recipients of transgenes, the applicant should provide information as to whether the level of toxicants is altered. If the plant produces no known toxicant, the applicant should state so and provide data or the reference to support the claim. Plant toxins can be assessed by the tests and criteria that plant breeders traditionally use in the crop. In some instances, this may be done qualitatively e.g., taste testing of cucurbits.

12.1.14 Assuming the levels of known toxicants in the regulated organism (plants) arein acceptable range; that there were no notable differences reported between transgenic and non-transgenic plant; and that the gene(s) engineered into the recipient plant have no known reported toxic properties; then, toxicological data on effects of the plant on non-target organisms and threatened and endangered species will usually not be required.

Important Information

Separate application should be submitted for each category/phenotype combination. For example, an application for insect-resistant cotton or PVY-resistant cotton should be submitted separately. However, when a single plant contains more than one phenotype modification, submit only one petition. For example, one petition should be submitted for cotton that are both virus and insect resistant.

C H A P T E R 13

SPECIFIC PROVISION TO GRANT EXEMPT STATUS

The IBC may award exempt status for Laboratory work/field working with Genetically Modified Organisms, if there is sufficient information/grounds available to consider the work as having no risk. Proposal/application for soliciting an exempt status is presented on the next page.

As in other cases, IBC assessment and project proposal shall be forwarded to the TAC/NBC for information only. The next page present a suggested format for preparing an application.

For those cases where IBC has granted exempt status for Laboratory work/field work with GMOs based on sufficient information/grounds available to consider the work as having no risks, the NBC may consider for formal approval for commercial release on priority basis. The NBC may notify the proposer within a period of 30 days.

IBC No.

National Bio-safety Committee (NBC)
Pakistan Environmental Protection Agency
Ministry of Climate Change &
Environmental Coordination
Government of Pakistan
Islamabad.

Cover Sheet

Project Proposal Form for Requesting Exempt Status

1	Name and full professional address of Principal Investigator submitting proposal.	2	Name(s) of other Principal Investigators responsible for the project. Please give their professional addresses if different from that in '1'.
	Tel: **Fax:** **Email**		**Tel:** **Fax:** **Email:**

3	Title of Project.

4	Project Objectives

5	**Methodology and Protocol (** *i. Provide through description of the main experimental procedures; ii. Include timetable of activities.*

6	Reasons why Project Merits Exempt Status

6	Signature of Principal Investigator submitting this proposal.
	Date / /

C H A P T E R 14

APPLICATION FORMAT FOR I M P O R T OF GENETICALLY MODIFIED ORGANISMS AND PRODUCTS THEREOF FOR FOOD, FEED AND PROCESSING (FFP)

When GMOs and/or products thereof have been adequately field tested and enough data accumulated to show that the experimental organisms and its products are free from any risk. An application may be made requesting that the said GMO or its products are safe and be allowed to considered for or crushing for food, feed and processing. The application/project proposal must contain sufficient detail, field performance data as well as relevant molecular biology data, to ensure that the reader would be able to ascertain that the material poses no risk. The proposal/application may be including data obtained elsewhere particularly by the technology developer himself or his nominated person/Lab/institute/country (not necessarily by the applicant) whether favorable or unfavorable to the position of "no risk status".

The succeeding pages present an updated format for preparing an application.

IBC No.

National Bio-safety Committee (NBC),
Pakistan Environmental Protection Agency
Ministry of Climate Change &
Environmental Coordination,
Government of Pakistan, Islamabad.

Application for Permission to Import Genetically Modified Organisms (GMOs)/Living Modified Organisms (LMOs) Intended forDirect Use as Food or Feed, or for Processing (FFP)

GMO PRODUCTS TO BE IMPORTED FOR USE AS:

☐ Food ☐ Feed ☐ Processing ☐ Food, Feed and Processing

COUNTRY OF ORIGIN (Make separate entry for each Country)	**NAME OF GMO PRODUCTS** Scientific or English names must be included (*colloquial names are* not acceptable) List of Products, by-product, and substances	**QUANTITY PER ANNUM (in metric tons)**	**PAKISTANI PORT OF ARRIVAL/ DISCHARGE**

Use additional pages if more space is needed and "X" box □

2	MEANS OF IMPORTATION/	Air Mail or Parcel Post [ ] A i r Freight [ ] Car/ [ ] Surface Mail or Parcel Post [ ] Truck, Rail, or Ship [ ] Baggage [ ]

	Name & Signature of Applicant			
CNIC: NTN/STN				

	ENCLOSURES	**ENCLOSED ("X")**	**IF PREVIOUSLY SUBMITTED LIST DATE & PERMIT/DIARY NO.**
a.	The name and contact details of the applicant for a decision to grant the import license/permit.		
b.	Names, addresses, and telephone numbers of developer/supplier/distributor/exporter of the products, by-product, and substances etc. of genetically modified organism (GMO) to Pakistan for the purpose of FFP.		
c.	Name and identity of the source GMO from which products, by- product, and substances etc. have been obtained.		
d.	Description of the source GMO including: i) gene ii) gene modification/s (GM)and/or GM event/s iii) Event Code iv) Trade Name v) Gene Source		
e.	Description of the technique used for the gene modification of the source GMO (if available with the developer/exporter/supplier)		
f.	Description of resulting characteristics/trait of source GMO.		

g.	Detail of any unique identification of the source GMO.		
h.	Taxonomic status, common name, and characteristics of recipient/parental organisms of the source GMO.		
i.	Center (s) of origin, IF KNOWN, of the recipient/parental organisms and a description of the habitats where the recipient/parental organisms may persist or proliferate.		
j.	Name of three (3) countries where approvals have been granted for use of this GMO trait/stack and the nature of approval in each of the countries [*e.g., for cultivation and/or for use as food, feed, or for processing (FFP)*		
k.	A risk assessment report of the source GMO which has been done at its country of origin. The risk assessment should have been evaluated and certified by the regulatory bodies of the country of origin of the source GMO.		
l.	Details of suggested methods for the safe handling, storage, transport, and use, including packaging, labelling, documentation, disposal, and contingency procedures.		
m.	Emergency response procedures that will be applied in Pakistan in the event of adverse consequences/ misuse of the product.		
n.	Details of the final disposition/use of the regulated article within purview of FFP		
o.	Name and Qualification of the Molecular Biologist/Biotechnologist/Agri-technologist who has prepared the dossier for the grant of the license.		
p.	Name of the Institutional Biosafety Committee (IBC) through which the dossiers of the application have been processed.		

* IBC may require additional details of gene(s), gene modification(s), genetic engineering processes, gene modification events, details of recipient/parental organisms of the source GMO, risk assessment and other data etc.

* In case of an ambiguity TAC/NBC may require samples and randomly perform/outsource (with justification) appropriate genetic identification test(s) to testify applicant's claims at the applicant's cost.

For more information, please refer to National Biosafety Guidelines 2005 (amended 2024), Pakistan Biosafety Rules 2005 (amended 2024) and Annexure II of Cartagena Protocol on Biosafety to the Convention on Biological Diversity.

CERTIFICATION

(To be submitted along with application on Rs.100 Stamp Paper)

I certify that the information given in this application is correct and I understand the consequence of giving false information, or violation of any terms of the import license for FFP shall subject the undersigned to:

(i) confiscation of the consignment;

(ii) penalty/fine under laws of Pakistan;

(iii) cancellation of the import license;

(iv) any other consequences under the laws of Pakistan.

Signed by applicant:

CNIC:_ _____

NTN/STN: _____

Date: _____

IBC No: _____

National Bio-safety Committee
(NBC) Pakistan Environmental
Protection Agency
Ministry of Climate Change &
Environmental Coordination,
Government of Pakistan, Islamabad.

IBC Permission

To Import GMOs for use as Food	To Import GMOs for use as Feed	To Import GMOs for Processing	Import of GMOs for use as Food, Feed, Processing

	IBC Review

	Specific Comments if any

Recommendation with/without Provisos
Signature of IBC Secretary
Date / /

14.1 THE APPLICATION MUST BE SUPPORTED BY THE FOLLOWING DATA/DOCUMENTS

1. Rationale for Development of the Organism/Product

2. The Organism or its Product
   a) Modified, Unmodified organism (e.g., Soybean, Maize, Mustard, and canola etc.)
   b) Taxonomy of the organism
   c) Genetics of the organism
   d) Mode of reproduction
   e) Characteristics of the unmodified organism/product If the organism is a crop. plant, please proceed to (f), (g), (h)and (i)
   f) Modes of gene escape in the environment
   g) Weediness (the organism is a crop or plant)
   h) Effect on Narrow or Broad leaf weeds
   i) Mode of action on Target and non-target insect pest

3. Description of the Modification/Transformation

4. Donor Genes and Regulatory Sequences
   a) Gene of interest
   b) Marker gene

5. Genetic Analyses of Field Performance
   a) Southern, Western and Northern Blot Analyses
   b) Expression/ELISA analysis of inserted genes
   c) Characteristics of the modification/improvement
   d) Characteristics of the specialized feature introduced as a modification.
   e) Mendelian inheritance
   f) Mycotoxins (in the case of plants)

6. Environmental Consequences of the Modification
   a) Specific advantages
   b) Current uses of unmodified organism/product, normal practices and disadvantages
   c) Proposed use of the modified organism/product with economic and social advantages
   d) Possible side-effects
   e) Effects on related organisms
   f) Vertical transfer of the new genes
   g) Horizontal transfer of the new genes

7. Adverse Consequences of the Modification

8. References

14.2 INSTRUCTION FOR THE PREPARATION OF THE APPLICATION FOR IMPORT OF GENETICALLY MODIFIED ORGANISMS AND PRODUCTS THEREOF FOR FFP

14.2.1 Description of the biology of the non-modified & modified recipient organism should include taxonomy, genetics, if the organism is plant, pollination, and evidence of reported weediness (e.g., noting whether the crop or sexually compatible species is listed in the relevant publications of the Weed Society of America) and discussion of sexual compatibility with wild and weedy free-living relatives in natural crosses or crosses with human intervention. The applicant should provide the source of recipient (cultivar name or accession number) and the weed status of its sexually compatible relatives.

14.2.2 The applicant should explicitly identify the lines to be considered in the application and the cultivars from which they are derived. If there are multiple lines, each line must be given a unique identifier and listed in the application. For virus-resistant plants, applicants should provide in an additional section on the nature of the virus that provided the sequences encoding the resistance phenotype:

i) The taxonomic name of the virus including family, genus, and strain designation including any synonyms;
ii) The type of nucleic acid contained in the virus;
iii) Whether the infection is systemic or tissue specific;
iv) Whether the virus is associated with any satellite or helper viruses;
v) The natural host range of the virus;
vi) How the virus is transmitted;
vii) If transmitted by a vector, the identity of the vector including mode of transmission (e.g., persistent or non-persistent);
viii) Whether any synergistic or trans-capsidation interactions with other viruses under field situations have been reported in the literature, and
ix) The location and the name of the host from which plant the virus was originally isolated.

The above information can be provided in a table format. This information can be Supplemented by listing references that report the host range, insect vectors, etc., for the virus.

14.2.3 For *Agrobacterium*-based transformation protocol, the applicant must indicate how Ti plasmid-based vector was disarmed (i.e., all tumorigenic DNA was removed). Applicants can provide citations that describe the transformation procedure. However, any significant modifications of transformation, strain designation, etc. should be described.

For other methods of transformation, the applicant can describe the sources of various components of the plasmid (or other DNA including possible carrier DNA) and method of transformation by citation. However, any significant modifications in transformation, tissue regeneration, etc. should be described.

14.2.4 The applicant must provide a detailed restriction map along with gene(s) name, gene host, insertion site of the gene(s) description and sequences of detection primers of the plasmid that is sufficient to be used in the analysis of Southern data. Description

of added restriction sites is helpful in interpretation of Southern data and should be provided.

14.2.5 In general, it is always prudent to analyze data statistically when such analysis is possible. When unpublished information or an opinion has been supplied by a scientific expert, a letter communicating the information should be included in the application. If the unpublished information provided is data resulting from scientific research, then these data can be provided as a personal communication either in a letter from the researcher or in the text of the project/application. In either case the materials and methods, data analysis, and discussion of the data analysis should be provided in detail. Unsupported assertions about the results of the experiment are not acceptable.

Applicants must report any differences noted between transgenic and non-transgenic plants that are not directly attributed to the expected phenotype. Differences observed could include changes in morphology, rates, other changes in overwintering capabilities, insect susceptibilities, diseases resistance, yield [if plants, pollen viability, seed germination and agronomic performance etc]. Applicants must also note the types of characteristics that were compared between unmodified and modified organism.

The applicant should describe whether data submitted are from inbred or hybrid plants; if hybrid
plants, state which generation.

If the organism is Plant, please address the following:

14.2.6 State whether data with respect to plant performance were generated in a greenhouse or field environment. If from the field, indicate how many sites, states and number of years the data represents. Seed germination, seed dormancy, seed production, growth rate, and other data relating to the plant's performance will be required when the nature of the gene and the biology of the plant (including sexually-compatible relatives) warrant such data. This type of data will usually not be required for plants that are highly domesticated (e.g., corn), are exclusively self- pollinating (e.g., soybean), are male sterile, and have high seed germination rates (>90%), and whose phenotypes are unlikely to affect performance with respect to weediness or fitness (e.g., delayed ripening or oil seed modification). Phenotypes that might require performance data (depending on the plant) include but are not limited to the following: could tolerance, salt tolerance and tolerance or resistance to other biotic or abiotic stresses.

14.2.7 Southern analysis should include DNA isolated from non-modified & modified recipient, all or selected transformed lines, and the vector. Parental plasmid DNA (e.g., PUC 18) not containing intended donor genes may be labeled and hybridized to Southern blots to demonstrate that only the intended sequences have been incorporated in the genome of the transgenic plant. Restriction enzymes to be used might include enzymes that do not cut within the transforming plasmid but will cut the entire insert into one fragment from the DNA of the transgenic plant.

In the case of an *Agrobacterium*-based transformation system, the applicant should determine if genes that reside outside the LB/RB are inserted in the genome of the regulated cultivar. If a complete copy of any of these genes is present, the applicant should determine

whether it is expressed in the plant. For direct transformation systems, applicants should determine which sequences are inserted in transgenic plants and whether they are expressed. PCR analysis may be used to prove that only the targeted DNA has been incorporated. Sequencing of the transgene in plant and adjacent sequences is not required. Determination of the number of copies of integrated transgenes is not required, but the number of insertions may be used to support analysis of inheritance data.

14.2.8 If the inserted DNA sequence order is complex, as is often the case for plants engineered via direct transformation systems (e.g., electroporation, polyethylene glycol transformation of protoplasts, or particle bombardment techniques), the applicants should summarize the data by providing the following information for the all genes (whether under the direction of plant or bacterial promoters), is there a complete copy of the gene present in regulated article? Is the protein expressed in the plant? If multiple complete copies of a gene are present, applicants do not have to determine if each copy of the gene is expressed. It is very helpful to provide a table, that summarizes the results and indicates where specific data is to be found.

14.2.9 Mendelian inheritance data and Chi square analysis for at least 2 generations are appropriate to demonstrate whether the transgene is stable inserted and inherited in Mendelian fashion. Such data are generally not necessary for infertile vegetatively propagated crops such as male-sterile potatoes.

14.2.10 RNA-Northern analysis is generally not required except for virus-resistant plants. However, such analysis may be necessary for ribozyme, truncated sense, or antisense constructs, when protein levels cannot be provided.

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For virus resistant plants, the amount of viral transgene RNA produced should be determined and compared to the amount of the RNA produced by the viral gene in an infected non- transgenic plant. Applicants should address whether the transgenic RNA (or protein) is present inthe same tissues as are infected during natural infections. In addition, provide the amount of bothcoat proteins (i.e., from the transgene and the naturally infecting virus) produced in the transgenic plant singly infected with the widely prevalent viruses in the U.S. that normally infect the recipient plant (contact NBC for the list of these viruses). For comparison, provide the amount of both coat proteins produced in the non-engineered plant in mixed infections of the virus from which the coat protein gene was derived and the same widely prevalent viruses used in the single infection study. Provide description of symptoms of infected plants in all cases.

14.2.12 For all diseases and pathogens surveyed, names of the diseases and the scientific names of the pathogens should be provided. Data from field tests in foreign countries are acceptable. If the data on diseases and pests were obtained in the foreign country, the applicant should submit information about the distribution of those pests; disease or pathogens in Pakistan or the sub-continent. Disease and pathogen susceptibility on wild type and transgenic plants should be determined preferably from natural infestations. However, if applicant must use direct

Inoculations; i.e., with virus resistant transgenic plant, the source and taxonomic classification of the virus should be provided.

14.2.13 Certain plants have minute quantities of known toxicants which may adversely impact nontarget organisms and beneficial insects; e.g., tomatine in tomatoes, cucurbit in in cucurbits, gossypol in cotton etc., if such plants are recipients of transgenes, the applicant should provide information as to whether the level of toxicants is altered. If the plant produces no known toxicant, the applicant should state so and provide data or the reference to support the claim. Plant toxins can be assessed by the tests and criteria that plant breeders traditionally usein the crop. In some instances, this may be done qualitatively e.g., taste testing of cucurbits.

14.2.14 Assuming that the levels of known toxicants in the regulated organism (plants) arein acceptable range; that there were no notable differences reported between transgenic and nontragic plant; and that the gene(s) engineered into the recipient plant have no known reported toxic properties; then, toxicological data on effects of the plant on nontarget organisms and threatened and endangered species will usually not be required.

14.3 *Special Consideration*

Following special consideration shall be followed while considering the import for FFP.

i. To stop the leakage of imported grains the importer will maintain the traceability record of imported grains consumption this will be added in the terms and conditions of license issued in respect of biosafety clearance.
ii. The importer/developer of technology will submit cases for deregulation of genetic events related to imported grains, to NBC accompanied by complete certified risk assessment data report generated in the country of origin.
iii. The certified risk assessment report by relevant agency in the country of origin for imported grains meant for Food, Feed and Processing will be acceptable in Pakistan. However, PARC may conduct retest in exceptional cases, if a need arises.
iv. PARC will screen the candidate lines of soybean submitted in National Uniform Yield Trial for GM Testing.

These guidelines are contingent upon the applicability of the revision in Pakistan Biosafety Rules 2005 (amended 2024) and if the Rules are not revised as per Sunset Clause, as mentioned in sub-rule 2(2A) of Rule 14 of S.R.O 45(1)(2024) dated 18th Jan 2024, these guidelines will become infructuous in consistent with the Pakistan Biosafety Rules, 2005 (amended 2024).

A P P E N D I X 1

GOOD LABORATORY PRACTICE

- Never do direct mouth pipetting of infectious or toxic fluid; use a pipettor.
- Plug glass and other pipettes with cotton.
- Do not blow infectious materials out of pipettes.
- Do not prepare mixtures of infectious materials by bubbling expiratory air through the liquid with a pipette.
- Use an alcohol moistened pledget around the stopper and needle when removing a syringe and needle from a rubber stoppered vaccine bottle.
- Use only needle-locking hypodermic syringes. Avoid using syringes whenever possible.
- Dispose excess fluid and bubbles from a syringe vertically into a cotton pledget moistened with disinfectant, or into a small bottle with a cotton pad.
- Before and after infecting an animal, swab the site of injection with a disinfectant.
- Sterilize discarded pipettes and syringes after use.
- Before centrifuging, inspect tubes for cracks.
- Use centrifuge bottles with tight lids.
- Wrap a lyophilized culture with disinfectant-wetted cotton before breaking. Always wear gloves and lab coat.
- Never leave a discarded tray of infected material unattended.
- Sterilize all contaminated discarded materials.
- Periodically, clean up deep-freezes and refrigerators in which cultures are stored to remove broken ampoules or tubes. Use rubber gloves and respiratory protection during the cleaning.
- Handle diagnostic serum specimens carrying a risk of infectious hepatitis with rubber gloves.
- Develop the habit of keeping your hands away from your mouth, nose, eyes and face. This may prevent self inoculation.

- Avoid smoking, eating and drinking in the laboratory.
- Make special precautionary arrangements for respiratory, oral, intranasal and intratracheal inoculation of infectious material.
- Give preference to operating room gowns that fasten at the back.
- Evaluate the extent to which the hands may become contaminated with some agents and operations, forceps or rubber gloves are available.
- Laboratory clothing should not be worn taken to the dining room, library and other non-laboratory areas.
- Decontamination of work surface is a must.
- Shake broth cultures in a manner that avoids wetting the plug or cap.

Aerosol Minimization

Because of their entrapping nature, pose special problems in that the laboratory worker may be unwillingly exposed. Procedures which produces aerosols include:

- Grinding
- Blending
- Sonicating
- Resuspending packed cells or viruses
- Inserting a hot loop into a culture
- Centrifugation
- Flaming an inoculation loop so that it break
- Forceful ejection of fluid from a pipette or syringe
- Releasing the vacuum on a freeze dryer
- Opening a tube within which the air pressure may differ from that of the room such as may occur when the tube is opened at a temperature different from which it was sealed.

APPENDIX 2

NBC AUTHORIZED HOST/VECTOR SYSTEMS

The National Biosafety Committee regards and evaluates host/vector systems primarily on the basis of the potential for the aggregate to survive and to multiply in the open environment or generally, the viability of the system in conditions that may be encountered beyond that of the source laboratory. These biological containment and general biosafety concerns also take into consideration the natural tendency for the vector(s) to be transferred to non-target hosts, whether the conditions be, among others, the laboratory setting, field test plots or the immediate surroundings.

Host/Vector Systems approved by the National Biosafety Committee on the basis of biological containment provided:

Class	**Host**	**Vector**
Bacteria	*Escherichia coli* K12, or a derivative thereof, which does not contain conjugative plasmids or generalized transducing phages.	1. Non-conjugative plasmids 2. Bacteriophage - lambda - lambdoid - F1 (e.g. M13)
	Bacillus subtilis or *Bacillus licheniformis*; Asporogenic strains with a reversion frequency of less than 10^{-7}	Indigenous Bacillus plasmids and phages with host ranges not inclusive of *B. cereus* or *B. anthracis*
	Pseudomonas putida stain KT 2440	Certified plasmids: pKT262, pKT 263 and pKT 264
	Certified Streptomyces species: *S. coelicolor* *S. lividans* *S. parvulus* *S. griseus*	1. Certified plasmids: SCP21, SLP1, SLP2, PIJ101 AND derivatives thereof 2. Actinophage phi C31 and derivatives thereof
Fungi	Specified strains of Neurospora crassa modified to prevent aerial dispersion	No restriction
Tissue Culture	Mammalian, including human, cells	Non-viral or defective viral vectors (including retrovirus or retroviral/helper combinanation) that cannot infect mammalian cells.
	Plant cell culture	Disarmed non-tumorigenic Ti plasmid vectors in *Agrobacterium* tumefaciens and non-pathogenic viral vectors.

Source: Guidelines for Small Scale Genetic Manipulation Work, January 1993, GMAC, Department of Administrative Services, Australia.

Researchers should be aware that endorsement of host/vector systems is only conditional and that the NBC reserves the right to withdraw, if having new, pertinent information or developments, its authorization of any host/vector system in practice.

Special Provision:

Situations in which the donor DNA is introduced into the host through electrical, mechanical or any other means without the use of biological vectors, shall be regarded and treated, by the IBC, MBC sand the NBC, as approved host/vector systems provided that all of thefollowing conditions are realized:

- The host represents any of the above approved host organisms or tissue cultures.
- The donor DNA is not derived from microorganisms which are root causes of diseases in humans, plants or animals.
- The donor DNA represents or comprises no more than 2/3 of any complete viral genome and is employed in such a manner as to disallow the possible regeneration of live viruses (as opposed to such work wherein the hosts carry the missing segments of viral genomes or whereby regeneration is made possible under the context of ensuring propagation sequences).
- The donor DNA does not code for proteins which regulate the growth of mammalian cells (e.g. product of oncogenes), for cytotoxic proteins, or for toxins, to vertebrate, with an LD50 of less than 100ug/kg.

Such systems may be considered for an exempt status under Category 1 work.

APPENDIX 3

HIGH-RISK, VIRULENT TOXINS

Laboratory genetic manipulation work which entails the cloning of gene sequences (or the breeding and propagation of microorganisms carrying such sequences) coding for toxins, to vertebrates, with an LD50 of less than 100ug/kg must be authorized by the National Biosafety Committee before any work is allowed under way. A list of some such lethal toxins follows:

- Abrin.
- Bacillus anthracis lethal factor.
- Cholera - see Vibrio cholerae
- Clostridium perfringens epsilon toxin.
- Clostridium tetani toxin.
- Escherichia coli heat labile (LT) enterotoxin and LT-like toxin.
- Oxygen-labile haemolysins such as streptolysis O.
- Pseudomonas aeruginosa exotoxin A.
- Ricin.
- Shigella dysenteriae toxin.
- Staphylococcus aureus determinants A, B and F, alpha and beta toxin, and exfoliative toxin.
- Vibrio cholera (comma) toxin and toxins neutralised by antiserum monospecific for cholera toxin (e.g. heat labile toxins of E. coli, Klebsiella and other related enterotoxins).
- Yersinia enterocolitica heat stable toxins.

Source: NIH Federal Register Vol. 51, No.88, May 1986 (Appendix-F) provided by the NIH Office of Recombinant DNA Activities. The NBC would appreciate any notices from researchers of other virulent toxins determined to have an LD50 of less than 100ug/kg. The NBC requests supporting data on such toxins in order to confirm and accredit the findings.

APPENDIX 4

REQUIREMENTS FOR CONTAINMENT LEVEL C1

A Note on Physical Containment

Containment Levels C1, C2 and C3 are the different classes or grades of physical containment which may be afforded by genetic manipulation laboratories/facilities. The fundamental objective of physical containment is to prevent undue exposure of the laboratory worker, the community or the environment to regulated material and as such, is achieved primarily through the adoption of proper laboratory procedures and containment equipment. Special laboratory design provides a secondary means of protection and figures prominently in the containment of the more hazardous, genetically manipulated agents.

Three standard combinations of laboratory procedures, containment equipment and laboratory design give rise to the three basic levels of physical containment, detailed hereinafter. Where alternative combinations or supplementary precautions may better address the concerns of research work at hand, the project supervisors and the IBCs involved shall be primarily responsible for the preparation of specific and varied provisions, as is appropriate (e.g. immunization requirements, inactivation of infectious wastes, provisions for a central vacuum systems).

Containment level C1 provides the lower level of physical containment, and relies on good laboratory practice as the crux of laboratory biosafety and security. Some essentialequipment is required but otherwise, basic laboratory design suffices. Operating procedures, for the most part, reflect good microbiological practices.

Laboratory Procedures

1. When work is in progress, laboratory doors are closed and entrance is restricted at the discretion of the project supervisor.

2. Personnel wear, laboratory coats or gowns which are removed before leaving the laboratory.

3. Eating, drinking, smoking and applying cosmetics are all prohibited.

4. Food and drinks may not be stored in the work area at any time.

5. Mouth pipetting is prohibited; mechanical pipetting instruments are used instead.

6. All procedures are performed cautiously to minimize the creation of aerosols. Use of sonication or vortex machines, and other procedures which tend to generate aerosols, should be done in biological safety cabinets.

7. Personnel wash their hands with liquid soap and warm water after handling experimental organisms and cultures, and before leaving the laboratory.

8. Instruments used in culture work or with contaminated material are to be disinfected after use, if the instruments are not readily steam sterilized. For glassware, a hypochlorite solution made up with 5000 ppm of chlorine (dilute household bleach 1:8 and allow to react for at least 30 minutes), prepared daily, provides a suitable disinfectant.

9. Floors, work benches and surfaces are decontaminated with a suitable disinfectant after each session and immediately after any spill of viable material. Other than the basic hypochlorite solution, disinfectants must be prepared and used according to the manufacturer's instructions.

10. All microbiological wastes are decontaminated (preferably, autoclaved) before disposal.

11. Regulated materials are packaged within securely-sealed double containment (section 5.1) before being removed from the laboratory to autoclaves or rooms and facilities elsewhere. There "seals" must be opened to allow for thorough penetration of steam during autoclaving.

12. An appropriate pest control programme is in effect, as supervised by a licensed pest control operator.

Please Note: All work to be performed in a C1-level laboratory must observe C1-level procedures regardless of whether the work involves genetic manipulation.

Containment Equipment

1. Biological Safety Cabinets are provided (particularly, where laboratory operations tend to generate considerable amounts of aerosol).

   As terms of reference, specifications for and use of biological safety cabinets shall comply with the following Standards:

   - Biological Safety Cabinets (Class I) for Personnel Protection
   - Biological Safety Cabinets (Class II) for personal protection: Addresses the problem of frontal airflow disturbances and decontamination.
   - Laminar Flow Biological Safety Cabinets (Class II) for Personnel and Product Production.

2. There is close access to a steam sterilizer.

Laboratory Design

1. A closet, for laboratory coats and gowns, is provided next to the exit.

2. Room and work surfaces are smooth, impervious and resistant to attack by standard acids, alkalis, organic solvents and moderate heat.

3. The laboratory can be easily cleaned and thoroughly decontaminated; gaps and spaces between room surfaces, benches, furniture and equipment are accessible for wipe down. Ideally, benches, furniture and equipment should be anchored and sealed to room surfaces. False ceilings should be avoided.

4. Laboratory windows that open are fitted with fly-screens.

5. The laboratory entrance is labeled with an official sign designating the certified level of containment (available from the IBC, pending certification), with the universal biohazard symbol and, when work is in progress, with a notice detailing the entry requirements and procedures. Names and contacts (e.g. postal address, telephone number) of the responsible authorities should be clearly indicated.

6. Signs are posted within the laboratory, outlining the appropriate operating procedures, contingency plans and instructions for upkeep and maintenance.

7. Freezers, refrigerators, liquid nitrogen tanks and other appliances for the storage of recombinant DNA or of manipulated genetic material are labeled with the universal biohazard symbol.

A P P E N D I X 5

REQUIREMENTS FOR CONTAINMENT LEVEL C2

Containment level C2 provides a moderate level of physical containment through an even blend of proper laboratory procedures, suitable containment equipment and special laboratory design.

Laboratory Procedures
(all of containment level C1, and the following.)

1. Persons enter the laboratory for cleaning, audits, repairs and other activities at the discretion of the project supervisor (or the biological safety officer) and only after laboratory surfaces have been properly disinfected.

2. Laboratory coats, gowns and protective clothing are placed in sealed bags or boxes (which may be readily steam sterilized), and brought to an autoclave for decontamination after each session and before laundering. Laboratory clothing shall not be worn outside the laboratory.

3. Sonication, Vortex and other machines/instruments that generate aerosols are kept and used only in biological safety cabinets.

4. Work surfaces and biological safety cabinets are decontaminated with formaldehyde gas after each session and after major spills of viable material.

5. Laboratories are inspected and serviced periodically. Screens, filters, ventilation and drainage systems are cleaned regularly.

Please Note: No other work must be done simultaneously with work requiring C2 levels of physical containment.

Containment Equipment
(all of containment level C1, and the following.)

1. Independent room exhaust fans are installed to achieve room pressure control. Exhaust fans must be equipped with a variable-speed drive and should be able to maintain a minimum air pressure differential of 50 Pascals. Discharge should be through a high- efficiency exhaust filter...

2. Exhaust filters are HEPA class, outfitted with a metal separator and rigged to supplementary prefilter, of the same specifications as replacement air filters (#3). Exhaust filters will have a modular, metal framework and will not make use of fluid or grease seals. As a guide, and performance audits must observe A.S. 1807.6 testing guidelines.

3. Channels to draw in replacement air are engineered with contractible apertures and high-efficiency filters that prevent back-flow. As a guide, filter specifications must comply with Australian Standards 1324 for Type 1, Class A or Class B models, and have at leasta 90% arrestant efficiency against Test Dust No. 2 under A.S. 1132.5 testing guidelines.

4. An airlock is provided at each access, to maintain a reduced laboratory air pressure during entry and exit. The basic design incorporates a pair of outward-opening doors, arranged in sequence so that a small chamber rests in between. Each door must be self- closing and fitted with a viewing panel. The outer door requires a security lock.

5. Manometers are installed to monitor air pressure drop across exhaust prefilters and a Magnehelic type differential pressure gauge is outfitted to measure room negative pressure. Ideally, climate-control switches, exhaust fan speed dials and replacement air aperture-adjustment controls should be affixed next to the gauge to support manual room pressure control.

6. Special protective clothing, head covers, overshoes, gloves, molded surgical masks and respirators are provided as required.

Laboratory Design
(all of containment level C1, and the following. ..)

1. The laboratory is isolated from and does not open onto public walkways.

2. Laboratory windows are closed and sealed; walls, ceilings and floors are substantially airtight.

3. Access to roof spaces above the laboratory, and to other enclosing or contiguous voids, is restricted so as not to unknowingly compromise structural integrity.

4. Room and work surfaces are resistant to attack by disinfectants, gases and other agents used in the laboratory.

5. Ventilation designs allow the laboratory to operate at a requisite room pressure of 50 Pascals below external air pressure, when all doors are closed; during entry and exit through airlocks, internal air pressure shall remain at least 25 Pascals below external pressure.

6. Fans, filters and ventilation shafts are positioned to facilitate inspection and performance checks.

7. Architectural and structural requirements accommodate the need for laboratory pressure to be maintained below external air pressure. An airlock is provided at each access. Laboratory surfaces and windows can withstand the variable air pressure load imposed by ventilation fans during all modes of operation.

8. The various sensing devices set off or sound an alarm to indicate loss of room pressure control.

9. A fan coil cooling system, using chilled water or a refrigerant as the cooling medium, is in place, where exhaust ventilation rates alone cannot sufficiently offset room heat loads. Care must be observed in setting up the systems to avoid airflow disturbances in front of biological safety cabinets.

10. The laboratory can be sealed-off to allow for decontamination of the entire room with formaldehyde gas. Ventilation shafts, exhaust ducts and replacement air apertures can be closed-off (e.g. by way of dampers and cover plates). Ideally, provisions for remote power switches should be made to allow for the safe generation of fumes.

11. Systems are in place to treat formaldehyde gas, generated for the decontamination of work surfaces and biological safety cabinets, to allow for safe discharge into the atmosphere.

A P P E N D I X 6

REQUIREMENTS FOR CONTAINMENT LEVEL C3

Containment level C3 offers a higher level of physical containment and necessitates much more rigorous conditions than does containment level C2. Additional engineering and architectural requirements is warranted--ranging from the installation of various and duplicate items of machinery inside the work area, to the special design of laboratory ventilation and drainage systems, to be separate from those servicing the rest of the complex. Laboratory performance would likely be extended to include directional airflow and emergency life-support. Further, provisions for personnel safety might involve advanced protective clothing (e.g.one- piece positive pressure suits) and chemical showers. Additional operating procedures must be adopted as well, complementing the equipment and construction and construct of C2-level facilities.

Institutions, with plans to support C3-level laboratories, must confer with the NBC to determine and fulfill the relevant and extensive requirements, as dictated by the nature of existing risks and concerns. No discrete criteria are given.

APPENDIX 7

REQUIREMENTS FOR BIOSAFETY LEVEL PH1 IN PLANT GLASS HOUSES

Containment in Plant Glass Houses

Biosafety levels PH1, PH2 and PH3 are the different classes or grades of physical containment which may be afforded by plant glass houses and green houses. Although emphasis is on the containment of experimental plants and plant materials, the extent of concern of experimental plants and plant materials, the extent of concern encompasses the need for containment of other regulated material involved as well (e.g. donor DNA, biological vectors, tissues cultures). As with physical containment in laboratories, containment in plant glass houses is achieved through the adoption of proper operating procedures, containment equipment and room design.

Biosafety level PH1 provides the lower level of containment but for the most part, conditions are suitable for Category 2 genetic manipulation work on whole plants. PH1-level plant glass houses may likewise accommodate Category 3 work with whole plants, but often require supplementary operational routines and special room design (including minor structural modifications) for such purposes.

Design and Equipment

1. Glass houses have concrete floors.

2. Windows and other openings along walls and ceilings (e.g. vents) are fitted with fine screens (standard: thirty gauge 30/32 mesh wire gauze).

3. Drainage conduits are engineered to deter rodents, insects and other pests.

4. An ante-chamber is required at each access, except for those that lead directly to another containment facility. Ante-chambers must offer substantial pest deterrence features (e.g. automatic pesticide spray/aerosol devices, supplemented with conventional stickily pest strips). The innermost door should open inwards and be self-closing. A closet, for protective clothing and apparel, is provided within.

5. Biological safety cabinets are provided, as specified for containment level C1.

6. The glasshouse can be easily cleaned and thoroughly decontaminated; gaps and spaces between room surfaces, benches, furniture and equipment are accessible for wipe down.

7. The entrance to the glasshouse is labeled with an official sign designating the certified biosafety level (available from the IBC, pending certification), with the universal bioharzad symbol and, when work is in progress, with a notice detailing the entry requirements and procedures. Names and contacts (e.g. postal address, and telephone number) of the responsible authorities should be clearly indicated.

8. Signs are posted within the glasshouse, outlining the appropriate operating procedures, contingency plans and instruction for upkeep and maintenance.

Operational Routines

1. When work is in progress, all doors to the glasshouse are closed and entrance is restricted at the discretion of the project supervisor. On the whole, entry is reserved for those whose presence is warranted in programme or support activities. Entry requirements are binding and IBC authorization must be obtained for access.

2. All doors to the glasshouse are kept locked during off-hours, to prevent inadvertent access.

3. Personnels wear, laboratory coats or grown which are removed before leaving the laboratory.

4. Eating drinking, smoking and applying cosmetics are all prohibited.

5. Food and drinks, for human consumption, may not be stored in the work area at any time.

6. All procedures are performed cautiously to minimize the creation of aerosols. Those procedures which tend to generate aerosols should be done in biological safety cabinets.

7. Personnel wash their hands with liquid soap and warm water after handling experimental organisms and cultures, and before leaving the glasshouse.

8. Instruments used in culture work or with contaminated material are to be disinfected after use, if the instruments are not readily steam sterilized. For glassware, a hypochlorite solution made up with 5000 ppm of chlorine (dilute household bleach 1:8 and allow to react for at least 30 minutes), prepared daily, provides a suitable disinfectant.

9. Floors, work benches and surfaces are decontaminated with a suitable disinfectant after each session and immediately after any spill of viable material. Other than the basic hypochlorite solution, disinfectants must be prepared and used according to the manufacturer's instructions.

10. All biological wastes (e.g. plant materials, tissue cultures), soil and soil substitutes and the containers of viable or contaminated material are inactivated or sterilized before disposal.

11. Regulated materials are packaged within securely-sealed double containment units before being removed from the glasshouse to rooms and facilities elsewhere. Viable plants and tissues shall only be delivered to a certified containment facility. Transfer to other institutions must be approved by the supervising IBC.

12. Experimental plants are treated as if they all incorporate foreign genetically-manipulated DNA, regardless of the true extent of genetic modification.

13. An appropriate pest control programme is in effect, as supervised by a licensed pest control operator. Signs of arthropod infestation must be monitored scrupulously, with particular attention on mites, which are too small to be effectively screened out. Experimental plants, whilst permitting, should be treated regularly with a systemic insecticide. The plant house itself, should be subject to a fumigation regimen, tailored to the existing concerns.

14. Glass houses are inspected and serviced periodically. Screens, filters, ventilation and drainage systems cleaned regularly.

Please Note: Plant glass houses/green houses designed, and intended to support genetic manipulation work, should not be used for purposes other than such.

A P P E N D I X 8

REQUIREMENTS FOR BIOSAFETY LEVEL PH2 IN PLANT GLASS HOUSES

Biosafety level PH2 provides a moderate to high level of plant containment, as appropriate to the needs and concerns of the work at hand. Although there are basic requisites, there is no real standard combination of room design, equipment and operational routines. This flexibility allows for a range of containment environments, specifically addressing the risks involved and the media by which these risks are borne. With this in mind, principal investigatorsand supervising IBCs should conduct thorough risk assessments before taking any further measures to accommodate plant research work requiring PH2-levels of containment.

The NBC certifies PH2-level plant glass houses on a case-by-case basis, considering the nature of work to be supported by the facility. Occasionally, upon reviewing project proposals for PH2-level glasshouse work, the NBC may decide that proposed PH2-levels of containment are excessive or extravagant, and recommend the alternative use of PH1 level facilities with additional, imposed constraints.

An index of the basic requisites for biosafety level PH2, follows:

Design and Equipment
(all of biosafety level PH1, and the following....)

1. The joins between structural components are sealed. Inasmuch as possible, bench tops and work surfaces are seamless as well.

2. Ideally, transparent panels are constructed of impact-resistant material (e.g. methyl-methacrylate, commercially licensed as Perspex) or reinforced glass. If ordinary window glass is to be used, a hail-stone screen or some other encasing is outfitted to shield the panels.

3. Air supply and exhaust ducts are fitted with fine screens (standard: thirty gauge 30/32 mesh wire gauze).

4. A wash basin or sink is provided in the ante-chamber or in the glasshouse, next to the entrance. Where a laboratory adjoins and leads directly to the glasshouse, the wash basin may be located in the laboratory, by the connecting passage way.

Operational Routines
(all of biosafety level PH1, and the following.)

1. Before or upon entering the glasshouse, personnel wash their hands with liquid soap and warm water in the wash basin, provided inside the ante-chamber or by the entrance.

2. In the ante-chamber, before entering the glasshouse, personnel wear special protective clothing (e.g. gowns/boiler suits), and put on head covers, gloves, molded surgical masks as necessary.

3. Protective clothing and apparel are removed upon leaving the work area and kept in a closet provided in the ante-chamber. They are decontaminated and laundered regularly, after each session.

4. Materials and equipment, to be brought into or out of plant glass houses, are treated to kill arthropods and arthropod larvae, and to destroy eggs and any other active or dormant stages of the arthropod life-cycle. Treatment of soil is not always practical, so use of soil should be avoided. Soil substitute, which may be readily decontaminated, must always be treated.

5. Whenever possible, use soil substitute for cultivation. Use of soil is discouraged.

A P P E N D I X 9

REQUIREMENTS FOR BIOSAFETY LEVEL PH3 IN PLANT GLASS HOUSES

Biosafety level PH3 offers a most rigorous level of containment in plant glasshouse work. To maintain such stringent requirement through the duration of work, a number of special engineering and architectural requirements are heavily warranted, as with laboratory containment level C3. Additional operating procedures and safety provisions should be adopted to complement the special design of glasshouse facilities and systems.

Along the same lines as biosafety level PH2, no discrete criteria are given for biosafety level PH3. Selection of room design, containment equipment and operational routines shall be consistent with the concerns exposed in initial risk assessments, but the following basic requisites must always be observed:

Suggested Design and Equipment
(all of biosafety level PH2, and the following.)

1. All transparent panels are constructed of impact resistant material or reinforced glass. Regular window glass is not used, even if provisions are made to outfit hail-stone screens or any other encasing.

2. Ventilation designs allow the glasshouse to operate at a requisite room pressure of 50 Pascals below external air pressure. Manometers are installed to monitor air pressure differentials and a Magnehelic type differential pressure gauge is outfitted to measure room negative pressure. These various sensing devices set off or sound an alarm to indicate loss of room pressure control.

3. Air supply and exhaust ducts are fitted with HEPA filters.

4. Drainage water from the glasshouse and ante-chamber collect in a central tank. Plant glass houses and ante-chambers have impervious floors and lower-walls, and watertight doors so that accidental spills and cleaning fluids may be made to drain into the central tank.

5. Ante-chambers are equipped with an autoclave.

6. Wash basins and sinks for hand washing may be operated by foot, by elbow or automatically. Disinfecting foot baths are provided before the internal ante-chamber doors, leading into the glass house.

7. Plant glass houses and ante-chambers can be sealed-off to allow for space decontamination or fumigation of the entire facility against microorganisms and arthropods.

8. Mechanical units, such as generators and water pumps, are positioned preferably outside the glasshouse to allow for maintenance and repair without entry into controlled areas. Ideally, those equipment which receive or process experimental materials or wastes from glass houses, should be specially engineered to stifle leaks and preclude escape of viable material. Otherwise, such equipment must be contained under airtight constructions, which may be readily decontaminated. An egress shall be designed to provide access for servicing.

Operational Routines
(all of biosafety level PH2, and the following.)

1. All materials and equipment, to be brought into plant glass houses, are disinfected, except where the experimental organisms are microorganisms. Under such circumstances, treatment is at the discretion of the project supervisor.

2. Pots, troughs and other plant containers may be underlained with a network of watertight trays which empty directly into the drainage system or placed on non-absorbant floor, clean regularly.

3. Plant glass houses and ante-chambers are fumigated against microorganisms after each session, and immediately against arthropods upon any sign of infestation.

4. Waste water collected in the central drainage tanks are treated to kill viable material (e.g. microorganisms, arthropod and plant materials) before discharge.

5. In the event of a power failure, entry into the glass house is prohibited until the situation is abated, and power is restored or special measures are taken to avoid escape of any transgenic organism etc.

A P P E N D I X 10

REQUIREMENTS FOR BIOSAFETY LEVEL C1A IN ANIMAL HOUSES

Containment in Animal Houses

Biosafety levels C1A and C2A are the two prevalent classes or grades of physical containment afforded by animal houses. Although emphasis is on the containment of experimental animals, the extent of concern encompasses the need for containment of other regulated material involved as well (e.g. donor DNA, biological vectors, tissue cultures). As with physical containment in laboratories, containment in animal houses is achieved through the adoption of proper operating procedures, containment equipment and room design.

Biosafety level C1A provides a moderate level of animal containment and embodies the rudimentary precautions/constraints for all animal house work, including the engineering of transgenic and infectious species.

Suggested Design and Equipment

1. The entire animal house is constructed of impervious or impermeable surfaces, which may be easily cleaned and disinfected.

2. Sills under doorways and egresses are sealed or screened to deter rodents.

3. Windows and other openings along walls and ceilings (e.g. vents) are fitted with fine screens (standard:60 x 40 swg mesh, 51% free area).

4. Drainage conduits are engineered to deter rodents, insects and other pests. Floor drains are protected with liquid disinfectant or water traps.

5. An ante-chamber is required at each access, if the animal house is free-standing. The innermost door should open inwards and be self-closing. A closet, for protective clothing and apparel, is provided within. (Please Note: ante-chambers may still be required even where access to the animal house is through a large-scale containment facility)

6. Doors to animal rooms open inwards and are self-closing.

7. Biological safety cabinets are provided, as specified for containment level C1, (Appendix-7).

8. Tanks, aquariums and other rearing vessels, for invertebrates and aquatic vertebrates, are outfitted with mechanisms to guard the water supply and discharge system against experimental organisms and their gametes.

9. The entrance to the animal house is labeled with an official sign designating the certified biosafety level (available from the IBC), with the universal biohazard symbol and, when work is in progress, with a notice detailing the entry requirements and procedures. Names and contacts (e.g. postal address, telephone number) of the responsible authorities should be clearly indicated.

10. Signs are posted within the animal house, outlining the appropriate operating procedures, contingency plans and instructions for upkeep and maintenance.

Please Note: specification for animal rooms, cages, tanks, aquariums and the like will vary with the species involved. Investigators are strongly encouraged to consult with the NBC in preparing appropriate caging or housing requirements.

Operational Routines

1. When work is in progress and when experimental animals are 'uncaged', all doors to the animal house are closed and entrance is restricted at the discretion of the project supervisor. On the whole, entry is reserved for those whose presence is warranted in programme or support activities. Entry requirements are binding and IBC authorization must be obtained for access.

2. All doors to the animal house are kept locked during off-hours, to prevent inadvertent access.

3. Personnel sign a logbook, indicating the date and time of each entry and exit.

4. In the ante-chamber, before entering the animal house, personnel wear special protective clothing (e.g. jumpsuits), and put on head covers, overshoes, gloves, and pads as necessary. Particular care should be taken to avoid being bitten, clawed or scratched. Molded surgical masks and respirators should likewise be considered, where there is concern over the inhalation of aerosols.

5. Protective clothing and apparel are removed upon leaving the work area and kept in a closet provided in the ante-chamber.

6. Eating, drinking, smoking and applying cosmetics are all prohibited.

7. Food and drinks, for human consumption, may not be stored in the work area at any time.

8. All procedures (particularly, change of bedding material and washing or hosing down cages and pens) are performed cautiously to minimize the creation of aerosols. Whenever possible, those procedures which tend to generate aerosols should be done in biological safety cabinets.

9. Personnel wash their hands with liquid soap and warm water after handling experimental organisms and cultures, and before leaving the animal house.

10. Instruments used in culture work or with contaminated material are to be disinfected after use, if the instruments are not readily steam sterilized. For glassware, a hypochlorite solution made up with 5000 ppm of chlorine prepared daily, provides a suitable disinfectant.

11. Floors, work benches and surfaces are decontaminated with a suitable disinfectant after each session and immediately after any spill of viable material. Other than the basic hypochlorite solution, disinfectants must be prepared and used according to the manufacturer's instructions.

12. Animal cages and pens are cleaned regularly and decontaminated after use. Bedding material and animal wastes from cages and pens are inactivated or sterilized, along with other biological wastes (e.g. animal carcasses, tissue cultures), before disposal.

13. Ideally, modified and 'pur' animals are kept apart and in separate cages, to prevent escape of transgenes.

14. Whenever possible, procedures which may provoke experimental animals should be avoided.

15. Regulated materials are packaged within securely-sealed double containment units (section 5.1) before being removed from the animal house to rooms and facilities elsewhere. Live animals and tissues shall only be delivered to a certified containment facility. Transfer to other institutions must be approved by the supervising IBC. Containers of viable or contaminated material are sterilized before disposal.

16. An appropriate pest control programme should be effected, as supervised by a licensed pest control operator.

17. A central register is maintained for daily activities, documenting the course of work and the housing or disposal of experimental animals.

18. Animal houses are inspected and serviced periodically. Screens, filters, ventilation and drainage systems are cleaned regularly.

Please Note: Experimental animals under genetic manipulation work, and their tissues, may not be taken and used for other purposes.

Special Procedures for Work with Invertebrates

Work with Invertebrates that crawl, jump or fly should observe the following, additional criteria:

1. Cages and rearing tanks are numbered and documented.

2. Measures should be in place to detect, trap, recapture and/or destroy escaped invertebrates. An electric insect control device may be appropriate.

3. Containers of ticks, mites and the like should be kept over trays of oil.

4. Flying or crawling arthropods are handled on a white, or a contrasting-colored tray, to readily detect signs of fight and escape.

5. Chilling methods are adopted to 'anesthetize' active arthropods, and lessen the risk of flight or escape.

Recognizing that many invertebrates are carriers of human pathogens (e.g. midges, mosquitoes and biting flies harbor dangerous arboviruses while soft ticks are carriers of Borrelia and triatomid bugs are vectors for trypanosomes), experimental species known to harbor infectious microorganisms need to be contained under a level that is appropriate to the severityof the diseases borne.

A P P E N D I X 11

REQUIREMENTS FOR BIOSAFETY LEVEL C2A IN ANIMAL HOUSES

Biosafety level C2A provides a one-step higher level of animal containment and incorporates much of the technicalities specified for laboratory containment level C2. Please refer to Appendix-8, Requirements for Containment Level C2, in reviewing the constraints and criteria described below.

Design and Equipment
(all of biosafety level C1A, and the following.)

1. The animal house is isolated from and does not open onto public walkways. Controlled areas are physically separated from other areas of the animal facility.

2. Doors to the animal house are fitted with a 'fire-escape lock' which requires a special key for entry, but opens outward freely from the inside.

3. An airlock is provided at each access, as specified for containment level C2, (Appendix-8).

4. Ventilation designs allow the animal house to operate at a requisite room pressure of 50 Pascals below external air pressure, when all doors are closed; during entry and exit through airlocks, internal air pressure shall remain at least 25 Pascals below external pressure. Independent room exhaust fans and adjustable replacement-air apertures support room pressure control.

5. Air supply and exhaust ducts are fitted with HEPA filters. Filters should be of medium grade, with a minimum arrestance efficiency of 95% against all particles above 5 micrometers. An initial coarse or roughing filter should be considered, where there is concern over the clogging of exhaust filters with animal hair, downing, shedding and feed dust.

6. Manometers are installed to monitor air pressure differentials and a Magnehelic type differential pressure gauge is outfitted to measure room negative pressure. These various sensing devices set off or sound an alarm to indicate loss of room pressure control.

7. Climate control systems are in place, where exhaust ventilation rates alone cannot maintain a suitable atmosphere for animal comfort/welfare. 'Split-type air-conditioners are preferred, where supplementary cooling is required, as the circulation of external air should be avoided.

8. There is direct or close access to an autoclave.

9. Wash basins and sinks for hand washing may be operated by foot, by elbow or automatically. Disinfecting foot-baths are provided before the internal ante-chamber doors, leading into the glasshouse.

10. The finishes on work benches and furniture are impervious and may be easily cleaned and disinfected.

11. The animal house can be sealed-off to allow for decontamination of the entire room with formaldehyde gas. Systems are in place to render the fumes safe for discharge into the atmosphere.

Operational Routines
(all of biosafety level C1A, and the following.)

1. Work clothing are decontaminated by autoclaving, and laundered regularly, after each session.

2. Drainage exits on the floor are plugged with an airtight stopper, when experimental animals are present.

3. Cages and pens are autoclaved, in ready for cleaning and washing.

4. Work surfaces and biological safety cabinets are decontaminated with formaldehyde gas after each session and after major spills of viable material.

5. All waste material is autoclaved prior to disposal.

6. Live experimental animals must not leave the controlled areas.

Please Note: No other work must be done simultaneously with work requiring C2A levels of animal containment.

A P P E N D I X 12

GLOSSARY OF TERMS

Abiotic	Physical factor in the environment (e.g., temperature, salinity).
Accessible ecosystem	The environment immediately accessible to an organism if it were to move or escape from a site, and more distant habitats in the contiguous environment into which the organism or its offspring can disperse.
Aerosol	Suspension in air of finely dispersed solids or liquids.
Amphotropic retrovirus	A retrovirus that will grow in the cells from which it will be isolated and also in cells from a wide range of other species.
Aneuploid	Bearing a number of chromosomes that is not an exact multiple of the haploid number typical for the species.
Antibiotic	A chemical produced by fungi or bacteria that kills or retards the growth of other microorganisms.
Biological diversity	The number of species and their relative abundance within a given area, including also the phenotypic and genetic diversity maintained within the populations of thesespecies.
Biological safety cabinet	Specially constructed cabinets that are designed to protect workers and the environment from dangerous agents, especially bacteria and viruses.
Bioremediation	The use of organisms to remediate an environmental problem.
Biotechnology	As processes using living organisms or parts of organisms to make or modify products, to improve plants or animals, or to develop microorganisms for specific uses, or as "the application of biological systems in organisms to technical and industrial processes".
Cascading	Progressing through a succession of stages, phases, or levels.
Cell	The smallest structural unit of living organisms that is able to grow and reproduce independently.

Chromosome	A structure in the cell, consisting of DNA and proteins, that carries the organism's genes.
Clone (noun)	A group of genes, cells, or organisms derived from a common ancestor and genetically identical.
Clone (verb)	To generate replicas of DNA sequences of whole cellsusing genetic manipulation techniques.
Competitive ability	Ability of an organism to compete with other organisms for necessary resources (e.g., food, light).
Competitive exclusion	Interaction between two species in which one species excludes the other from use of resources within a habitat; can lead to extirpation or extinction.
Competitive potential	The ability of an organism to compete with other organisms for necessary resources. See "competitive ability".
Conjugation	A form of gene transfer from one bacterial or yeast cell to another. The transfer is accomplished by products of genes located on a small circular DNA molecular called a plasmid. The process of conjugation is found in nature and also used in genetic engineering.
Conjugative plasmid	A plasmid that codes for its own transfer between bacterial cells by the process of conjugation (mating).
Construct (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 reducedability 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 agent (does not necessarily result in sterility).

DNA	Deoxyribonucleic acid, the molecule that carries the genetic information for most organisms; consists of fournitrogenous bases and a sugar phosphate backbone.
Donor	The organism or cell from which DNA is derived for insertion into another organism (the host).
Donor DNA	Heterologous DNA segments to be combined with vector. Heterologous DNA refers to DNA derived from organisms taxonomically different from their host cells.
Ecological competence	Ability to survive, grow and reproduce in a specific habitat, and to maintain population numbers under ambient conditions of predation, competition, disease, and disturbance.
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.
Environment	An ecosystem or habitat, including humans and animals, which is likely to come in contact with a released organisms.
Environmental safety	The condition of being safe from environmental risk, detriment, or danger.
Epistasis	An interaction between genes in which one gene affects the expression of another.
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.
Eukaryotic	Belonging to the group of organisms whose cells contain a true nucleus. Eukaryotic organisms include animals, plants, and fungi.

Exotic	Introduced (i.e. non-native).
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.
Fecundity	The number of female offspring produced per average female in the population, or the number of offspring produced per individual.
Field trial	An experiment or trial that is performed outdoors or in an uncontained environment.
Fitness	In population and evolutionary biology, the success in survival and reproduction of an individual organism, a population, or a species, relative to other individuals, populations or species; the number of offspring that survive to reproduce.
Fragmentation	Asexual reproduction by detached parts, pieces, or structures of an organism.
Fusion	Joining of the cell membranes of two cells to create a daughter cell that contains the genetic material from both parents cells.
Gamete	A reproductive (egg or sperm) cell.
Gene	A hereditary unit of nucleic acid that specifies the structure of a protein or RNA molecule.
Gene flow	The exchange and movement of genes within and between populations and species.
Gene introgression	Incorporation of a gene into the gene pool of a population.
Gene therapy	The replacement of a defective gene in a person or other animal suffering from a genetic disease.
Genetic engineering	Any condition in which the original genetic expression of a genome undergoes spontaneous changes that alter the genome itself and (possibly) the traits expressed in its phenotype.
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.

Genetically engineered food(s)	Foods, food ingredients, and food additives produced through recombinant DNA techniques.
Genetically Modified Organism (GMO)	Living cells or organisms whose genetic material has been altered or modified by the enormous variety of techniques of modern molecular biology to make them capable of producing new substances or perform new functions.
Genome	The total genetic complement of a given organism.
Growth factor	A protein that stimulates cell division when it binds to its specific cell-surface receptor.
Hazard	The potential of an organism to cause harm to human health and/or the environment.
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 to enable production of proteins or further quantities of the DNA.
Host Cells	Living cells into which rDNA molecules are introduced.
Host range	All the possible organisms capable of harboring a specific organism.
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.
IBC	Institutional Biosafety Committee.
In vitro	Literally in glass; performed in a test tube or other laboratory apparatus.
In vivo	In a living organism.

Indigenous	Originating and growing or living in a particular geographic region or locale.
Indirect interactions	Effects of one organism on (an) organism(s) in the accessible ecosystem that occur through mechanisms involving abiotic factors or additional species. Examples include, but are not limited to: (1) modification of the physical environment affecting its suitability as habitat for another species, and (2) cascading effects of altered trophic function in a biological community of multiple species.
Infectious	Capable of invading a susceptible host, multiplying in it, and causing an altered host reaction ('disease').
Infectious material	Any living stage of an organism or any infectious or agent substance(s) that can cause disease in other organisms or parts thereof.
Interference	Non-reproductive functions of another organism or species, e.g., through changes in competition, predation, parasitism, etc.
Interspecific hybrid	An organism produced by mating between individuals of different species.
Interspecific hybridization	Mating(s) between individuals of different species that result(s) in the production of viable offspring.
Jumping genes	See transposons.
Lateral transfer	Exchange of DNA and the genes it codes for directly from one organism to another rather that the vertical transfer from parent to offspring. Also called horizontal transfer, it is common among bacteria, but has also occurred in higher organisms. Plasmid-mediated conjugation, bacteriophage-mediated transduction, as well as transformation in bacteria are well-known forms of lateral transfer. Transposons (see below) are suspected of causing lateral transfer in higher organisms.
LD50	The dose of a toxin or infectious agent that will kill one half of a population of organisms.
Life history	The developmental history of an organism fromfertilization to death, including all changes in physiological, behavioral, and reproductive characteristics.

Marker sequence	A DNA sequence introduced into an organism for the purpose of unambiguously identifying the specified individuals or their progeny.
Metabolism	The physiological process that allows organisms to obtain the energy and materials necessary for development, growth, and reproduction.
Microorganism	An organism that can be seen only with the aid of a microscope.
Monoclonal antibody	An antibody that is derived from a single clone of hybridoma cell and recognizes only one antigenic site.
Morphology	The physical appearance of an organism, including its form and structure.
Mutagenesis	Natural or artificial procedures that cause mutations in organisms; used to create mutant organisms in research and biotechnology.
Mutation	A structural change in a gene or chromosome that alters the genotype and possibly the phenotype of an organism. Examples of mutations include base-pair changes,deletions, insertions, fusions, and chromosomal rearrangements.
NBC	National Biosafety Committee
Negligible number of escapees	The number of escapees that is so small as to cause negligible biological or environmental consequences.
New species	A single distinct kind of plant, animal or microorganism having certain distinguishing characteristics as determined by biological classification.
Nitrogen fixation	The ability of some bacteria to remove elemental nitrogen from the atmosphere or water convert it to nitrate, the form of nitrogen that is an essential nutrient for most forms of life.
Non-coding DNA sequences	Some of these are DNA sequences that serve as spacer regions (introns) between sequences that are parts (exons)of a complete protein coding sequence; they are spliced out of the message (mRNA) that provides a cell with complete instructions for assembling the protein. Other non-coding sequences come in a variety of longer and shorter repetitive forms; no cellular function is known for any of them.

Non-indigenous species	Any species or viable biological material that enters ecosystems beyond its original range, including any such organism transferred from one region or country to another.
Non-reproductive	Interference by one organism or species in the
Non-target (non-target)	Not intended to be affected by a process,
Novel trait	Expression of a phenotypic (observable) trait not normally found in the species.
Oncogene	An activated (modified) cellular gene that causes normal cells to become cancerous.
Oocyte	A cell that divides to form the female reproductive cell.
Organism	Any entity able to replicate its own genetic material technique, or event.
Packaging	In the process of virus replication, the assembly of the components of the virus to form the complete virus particle.
Parasitism	Relationship between two organisms in which one organism (the parasite) derives benefit by growing in or on another organism (the host), and in which the hostorganism either derives no benefit or is harmed.
Parental organism(s)	The organism(s) to be used as parents in crossbreeding, or the initial organism which is the recipient of introduced genetic material, or whose genome is to be altered by addition, removal, or rearrangement of genetic material.
Pathogen	An organism that causes disease.
Persistence	The ability to continue through time.
Pest	Any living stage (including active and dormant forms) of insect, mites, nematodes, slugs, annelids, snails, protozoa, or other animals, bacteria, fungi, other parasitic plants or reproductive parts thereof; viruses; other plants and animals that can damage aquatic and terrestrial ecosystems; or any infectious agents or substances which can directly or indirectly injure or cause disease or damage in or to humans, plants or animals or any processed, manufactured, or other products of plants or animals.

Phenotype	The observable physical or biochemical characteristics of an organism as determined by both its genetic make-up and environmental influences.
Photosynthetic	The ability of some bacteria, algae and green plants to use sunlight as a source of energy to create the carbon-based organic chemical compounds essential for life.
Physical 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.
Planned release	Intentional release of Genetically Modified Organisms into the open environment.
Plant	Any living stage or form of any member of the plant kingdom including, but not limited to, eukaryotic algae, mosses, club mosses, ferns, angiosperms, gymnosperms, and lichens (which contain algae) including any part (e.g. pollen, seeds, cells, tubers, stems) thereof, and any cellular component (e.g. chloroplasts, mitochondria, ribosomes, etc.) thereof.
Plasmid	A small, self-replicating molecule of DNA that contains a specific origin of replication. Plasmids are often used as cloning vectors.
Project Leader	A person who will implement the planned release activities.
Prokaryote	Characterized by the absence of a nucleus, nuclear membrane, and other membrane-bound organelles. Includes bacteria and cyanobacteria.
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.
Proponent	May be an institution or person who will be responsible for all the planned release activities.
Protein	A molecule composed of amino acids.
Protein-encoding DNA sequences	These are either single stretches of DNA coding for a single protein, or parts of a complete protein-coding sequence that is spliced together by removing intervening (intron) sequences and joining the coding parts (exons) when the final RNA message is formed to provide instructions for the exact amino acid sequence of a given protein.

Protoplast	A plant or bacterial cell that has had the outer cell wall removed.
rDNA microorganisms	rDNA organisms whose host cells are microorganisms (including microalgae and fungi, except fungi that from sporophores). rDNA organisms that are used as undifferentiated cells, and whose host cells are animal or plant cells, are regarded as rDNA microorganisms.
rDNA molecules	Vector deoxyribonucleic acid (DNA) molecules combined with donor DNA.
rDNA organisms	(1) host cells into which rDNA molecules have been introduced (except living cells possessing the same genetic structure as that of naturally existing cells), or (2) cells or organisms derived via rDNA techniques from the living cells described in (1) above.
rDNA plants	rDNA organisms (excluding rDNA organisms that are used as undifferentiated cells) whose host cells are plants (excluding microalgae and fungi, except those that form sporophores).
rDNA small laboratory animals	rDNA organisms (excluding rDNA organisms used as undifferentiated cells) whose host cells are animals (restricted to mice, rats and other rodents) that are used as laboratory animals.
rDNA techniques	Methods by which to construct rDNA molecules by enzymes in vitro and to introduce the rDNA molecules into host cells in order to propagate donor DNA in the host cells.
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, cell, 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.

Regulatory DNA sequences	Gene sequences that do not code for proteins that go into the structure or metabolism of an organism. They serve to turn other genes on or off, or they increase or decrease the activity of protein coding genes, yielding more or less production from these genes. It is often said that these kinds of genes control or regulate the expression of other genes.
Remediation	A process by which damage is fixed, repaired, or returned to an original condition.
Replication	Reproduction.
Resiliency	Is the ability (of an ecosystem) to recover to a previousstate or conditions after a major change or disturbance.
Resistance/resistant	The ability of either organisms or enzymes to counter the effects of toxic materials or disease or harmfulenvironmental agent(s). Examples are resistance to malaria, or to antibiotics, insecticides, herbicides, orpoisonous metals such as mercury, lead and cadmium. An organism or enzyme which exhibits resistance is said to be resistant.
Restriction enzymes	Naturally occurring enzymes that cleave DNA molecules at specific sites to produce short fragments.
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.
Risk	The combination of the magnitude of the consequences of a hazard, if it occurs, and the likelihood that the consequences will occur.
Risk assessment	The process of identifying hazards to human health and environment that may be caused by human activity, including the process of assigning magnitudes and probabilities to possible adverse effects of such activity.
Risk management	The measures to ensure that the production and handling of organisms are safe.

RNA	Ribonucleic acid, a molecule similar to DNA whose function include decoding the instructions for protein synthesis that are carried by the genes; comprises the genetic material of some viruses.
Selection pressures	Natural or artificial force that favors survival of one individual or group over another individual or group in the same environment or ecosystem.
Somatic cell	Any cell of a multicellular organism other than germline cells.
Species	A group of organisms and populations that shares common genetic and phenotypic properties and are capable of interbreeding.
Sterilization	Act or process that kills or removes all infectious agents; applied particularly to bacteria and molds, their spores, and viruses.
TAC	Technical Advisory Committee
Target (target organism)	Intended to be affected by a process, technique, or event.
Tissue culture	In vitro growth of tissue cells in nutrient medium.
Toxin	A poisonous substance, produced mainly by microorganism but also by some fungi, plants, and animals.
Transcription	The synthesis of messenger RNA from DNA.
Transduction	A form of gene transfer among bacteria (found in nature and also used in genetic engineering). The transfer is accomplished by a bacterial virus called a bacteriophage (or just phage). After the bacteriophage has replicated (copied itself) numerous times within its host bacterial, cell, it forms protein wrapped viral particles containing its own DNA and often some parts of the host and donate the chromosomal DNA sequences to the new host, often changing the genetic makeup of the new host.

Transformation	A form of gene transfer among, for example, bacteria; the process is found in nature and also used in genetic engineering. During transformation, one bacterial cell copies its DNA and releases the copy into the environment, or it dies and its DNA becomes free in the environment. Another cell takes in the free DNA and with some frequency exchanges it for the same region of DNA in its own chromosome. If the process brings in different (variant) forms of the genes, the receiving cell is said to be transformed. An example would be the substitution of a gene for antibiotic resistance for its susceptible counterpart.
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.
Transgene	The foreign DNA to be transferred to an organism.
Translation	The process through which messenger RNA directs protein synthesis.
Transmissibility	The ability to be transmitted; in this case, the ability of an organism to transmit a disease to a range of organisms.
Transposons	Small DNA molecules that can move in and out of specific positions within the same chromosome or another chromosome of the same or different cell or plasmid. In moving, they may or may not leave a copy of their DNA base sequence behind. They are some times referred to as "jumping genes" or "selfish DNA". They are not well understood, but it is certain that they sometimes cause mutations.
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).

Work area	A location where rDNA organisms are handled directly.
Work site	Site where the characteristics of rDNA organisms are produced or evaluated. A work site comprises work are as defined in 8. above and other sites where rDNA organisms are not handled directly.
Xenotropic retrovirus	A retrovirus that is endogenous to a species but cannot replicate well in that species, generally because of a receptor back. Xenotropic retroviruses tend to have a wide range for replication in cell of heterologous species.
Zygote	The cell produced by the union of the male and female gametes.