Drafts for Amendment

Biosafety Guidelines of Bangladesh, 2020

গণপ্রজাতন্ত্রী বাংলাদেশ সরকার
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নং-বিতযোপ্রম/সা-১২/-১/২০০৫/১৪৪(২০)-বিগত ০৬/০৮/২০০৬ তারিখে অনুষ্ঠিত বাংলাদেশ জীবপ্রযুক্তি সংক্রান্ত টাস্কফোর্স (NTBB) এর ১ম সভায় (অনুচ্ছেদ ৮ এর সিদ্ধান্ত অনুযায়ী) Bio-Safety Guidelines Bangladesh অনুমোদিত হইয়াছে। যাহা নিম্নরূপঃ

CHAPTER-I

SCOPE AND OBJECTIVES OF BIOSAFETY GUIDELINES

1.0 Introduction

Bangladesh is interested in exploring the use of biotechnology to advance national development, while simultaneously recognizing the need for regulation to ensure the safety of the environment as well as human and animal health. The Biosafety Guidelines of Bangladesh are intended to provide a framework, legally binding under the Biosafety Rules of Bangladesh, to permit the development of biotechnology in accordance with a biosafety review to assess any risks to the environment as well as human and animal health. This advances the national policy and meets obligations that Bangladesh holds as a signatory to the Convention on Biological Diversity and the Cartagena Protocol on Biosafety.

Biotechnology can be broadly defined as the manipulation of living organisms or their components to produce useful products. Traditional biotechnology includes many activities such as fermentation, plant and animal breeding, and other biological processes used in the production of foods. However, the use of increasingly sophisticated biological science to manipulate living organisms has led to a distinction between different methods of developing and using biotechnology. The Cartagena Protocol on Biosafety defines "modern biotechnology" as the application of: a. In vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or b. Fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection. The products of modern biotechnology are the subject of the Biosafety Guidelines.

1.1. Scope of Biosafety Guidelines

Biosafety guidelines are applicable to all research and development activities of modern biotechnology as defined in the Cartagena Protocol on Biosafety conducted in laboratories of the government research institutes, state enterprises, universities, international organizations located in Bangladesh, private companies or non-governmental organizations. It applies to laboratory and field trial, trans-boundary movement, transit, handling and use of all GMOs that may have

adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health.

1.2. Activities not regulated by the Guidelines

The following activities are excluded from the guidelines:

(a) Research, development and promotional or commercialization activities involving organisms within the country that result from natural reproduction or micro propagated products derived through tissue culture or the use of traditional breeding practices.
(b) The movement of non-living products of modern biotechnology such as isolated nucleic acids or proteins or lyophilized or other killed biological materials for the purpose of research and development.

(c) Such other activities that may in future be declared to be excluded by the National Committee on Biosafety (NCB).

1.3. Objectives of Guidelines

In accordance with the precautionary approach contained in Principle 15 of the Rio Declaration on Environment and Development, the objective of this Guideline is to ensure an adequate level of protection for the environment and human health during the conduct of contained research, field trials, safe transfer, handling, use and trans boundary movement of GMO as part of modern biotechnology that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human and animal health.

Comment [u1]: Best to use uniform language throughout

On the basis of the precautionary principle, the Guidelines provide a framework for the following aspects:

(a) Provide the framework to ensure safety of the developers and end-users of modern biotechnological products.

(b) Promote the development and enforcement of regulations in harmony with national priorities and international obligations.

(c) Foster a favourable climate for developing and accelerating innovation and for adopting sustainable biotechnology products and processes.

(d) As necessary, develop materials in support of the objective of ensuring the safe handling transit and use of GMOs, including standard operating procedures, standards of best practice for record keeping and data recording, monitoring and inspection of regulated activities or other such guidance.

These guidelines covers aspects of risk assessment and safety requirements needed for undertaking (a) Laboratory work, (b) Field trial and (c) Commercial use, involving i) Microorganisms, ii) Plants, iii) Fish and aquatics and iv) Animals. Guidelines for laboratory work specify the experiments to be categorized as belonging to different biosafety levels reflecting work bearing minimal risk, work bearing low risk, work bearing considerable risk and work bearing high risk and what precautionary measures should be taken to avert such risk.

1.4. Review of the Guidelines

The Biosafety Guidelines have been developed on the basis of information presently available and since the field of modern biotechnology is fast advancing, the Guidelines will be reviewed as and when required to include safety aspects in the light of new developments in this field.

CHAPTER II

INSTITUTIONAL ARRANGEMENTS

2.0 Biosafety Committees

Ministry of Environment and Forest (MOEF), being the competent national authority and national focal point to implement Cartagena Protocol on Biosafety to the Convention on Biological Diversity (CBD), shall establish a National Committee on Biosafety (NCB) in order to ensure environmentally safe management of modern biotechnological development including research and development, introduction, use and trans-boundary movement of GMOs/LMOs. A Biosafety Core Committee (BCC) shall be working to assist and accelerate the functions of NCB. In order to ensure safe management of Biosafety activities in the laboratories and in the field there shall be committees under NCB, such as Institutional Biosafety Committee(IBC), Field Level Biosafety Committee (FBC) and also there will be designated Biological Safety Officers (BSO) in each research establishment of the country. The composition, functions and responsibilities of these committees are given in the following subsections:

2.1 National Committee on Biosafety (NCB)

2. 1.1 Composition of NCB

The NCB shall be composed of members as shown below. The scientists and or experts will be nominated by the Ministry of Environment and Forest. The structure of the committee will be as follows:

Chairperson:

1. The Secretary, Ministry of Environment and Forests
2. Full time Member Secretary to be nominated by MOEF.

Members:

3 Secretary, Ministry of Science and Technology or his designated nominee not below the rank of Joint Secretary
4. Secretary, Ministry of Agriculture or his designated nominee not below the rank of Joint Secretary
5. Secretary, Ministry of Fisheries and Livestock or his designated nominee not below the rank of Joint Secretary
6 Secretary, Ministry of Health and Family Planning or his designated nominee not below the rank of Joint Secretary
7. Executive Chairman, Bangladesh Agricultural Research Council.
8. Director General, Department of Environment.
9. Director General, Department of Agricultural Extension.
10. Member- two, Public Sector University Research Scientist to be nominated by MOEF.
11. Member- two, Public Research Sector Scientist to be nominated by MOEFCC (One Genetic Engineering, one Biosafety Affairs).
12. One representative from the NGO working with environment related issues to be nominated by MOEF.

Comment [SH2]: This is the highest decision making regulatory body in the country. The existing structure of the committee is constituted of the majority of the developer institutions that goes against the spirit of regulatory system. To avoid conflict of interest in the regulatory system and decision making number of proponent institutions reduced.

2.1.2 Tenure of members of the NCB

The term of office of the members shall be co-terminus with their holding of the posts. However, the terms of the MoEFCC nominated members shall be valid for three years, and may be re-nominated by MoEF.

2.1.3 Meetings of the NCB

The committee shall normally meet quarterly, unless the Member Secretary indicates the committee has no agenda for that quarter. If the chairperson desires, the committee may hold special meetings to consider urgent matters. The quorum of NCB meeting shall be formed with the presence of a majority (7) of the members.

2.1.4 Adoption of resolutions, guidelines or policies

The endorsement of majority members shall be needed for adoption of resolution, guidelines or policy decisions.

2.1.5 Honorarium to the NCB members

The members shall be entitled to Honorarium for their work as determined by the Ministry of Environment and Forest.

2.1.6 Powers, functions and responsibilities of the NCB

2.1.6.1. Powers

a. The NCB will draft and adopt policies and measures to ensure safety of humans and environment;

b. The NCB may stop or prevent any activity with a GMO after establishing that the activity is unsafe to the personnel, community and/or the environment;

c. The NCB shall approve applications for GMO research, introduction, commercial use, tranboundary movement and release to the environment;

d. Approve biosafety related guidelines, manuals, SOPs, and formats/forms;

e. Certify/Authorize laboratories to conduct GMO research; and

f. Approve Institutional Biosafety Committee (IBC) upon recommendation of respective heads of institutions/ universities/ private organizations.

2.1.6.2 Functions

a. Formulate, review, update or amend national policies, Acts and Rules on Biosafety of GMOs,

b. Review, monitor and recommend measures to minimize potential risks that may result from, import, contained use, field trial and release or introduction of new species, strains or varieties of GMOs.

c. Facilitate the institutes/faculties/companies already working with GMOs to obtain necessary permission or clearance in favor of their activities.

d. NCB shall examine the application submitted by any university, department and division of a research institute or a private company within a specified time frame and shall approve or disapprove the application on case-by-case basis within 90 days. MoEFCC will notify the decision of NCB to the applicant.

e. Ensure implementation of biosafety measures at laboratory, on-site (field), during trans-boundary movement, use, handling and release in the market.

f. Hold public deliberations on national policies, guidelines and other biosafety issues.

g. Identify priorities in human resource development in Biosafety and in the related capacity building (including infrastructural) requirements.

h.

i. NCB can co-opt relevant experts/officers as and when it will be required for.

j. Monitoring of all ongoing GMO related activities/programmes.

2.1.6.3. Responsibilities

a. Approve all major policy documents related to biosafety in Bangladesh, guidelines for food safety and environmental risk assessments

b. Issue approvals for wide-scale or commercial environmental release of GMOs that are determined to be safe for human health and the environment based on a technical review provided by the BCC

c. Make record of decisions and approvals available through appropriate forums.

d. Review the routine decisions made by the BCC in relation to laboratory and field trial of GMOs

e. Approve publication of support materials to explain biosafety processes and assist applicants in complying with these Biosafety Guidelines and other obligations under the Bangladesh Biosafety Rules

f. Advise relevant authorities to develop adequate technical, legal and institutional capacity on provisions of all pertinent internationals agreements.

g. Provide advice and assistance to the IBCs and FBCs and other relevant committees on the risk and safety aspects of their work.

h. Publish various forms/formats for application, assessment and evaluation, Biosafety Guideline and other documents to relevant committees in different organizations.

i. Inform institutions/ universities/ organisations engaged in GMO work about new developments in biosafety to avoid unnecessary risk.

j. Maintain confidentiality of information of commercial importance upon request from the researcher.

k. Consult, as appropriate, with other national bodies and institutions dealing with the safety of human being and the environment and inform these bodies all conceivable developments in the field of modern biotechnology.

l. Cooperate with other national authorities dealing with import of live organisms, to formulate uniform guidelines for identification, inspection and regulation of transgenic species, exotic organisms and others.

m. Advise various research institutions and regulatory agencies in setting up appropriate experimental conditions for genetic manipulation work avoiding potential hazard.

2.2 Biosafety Core Committee (BCC)

The Biosafety Core Committee is a technical committee supporting the NCB.

2.2.1 Composition of BCC:

The Biosafety Core Committee (BCC) shall be composed of eight members. The structure of BCC shall be as follows:

1. Convener- Director General, Department of Environment,
2. Member Secretary- One of the Directors Department of Environment having relevant expertise on biosafety and Biodiversity.
3. Members - Four Bio-scientists to be nominated by MOEF
4. Member -One Medical Scientists (Knowledgeable and experienced in human health aspects)
5. Member- One Scientist specialized in Biosafety affairs.
6. Director General, National Institute of Biotechnology.

Note: Bio-scientists will be chosen from the Public Universities and Research Institutes with expertise in relevant fields, such as the following areas: Environmental Safety, Foodsafety, Nutrition, Molecular Biology, Biochemistry, Plant, Fish and Animal Breeding, Microbiology, Entomology, and Plant Pathology; etc.

2.2.2 Functions of BCC:

The BCC shall perform technical functions in support of the NCB, including the following:

a. Monitor the implementation of Biosafety policies, guidelines, acts and rules on behalf of the NCB.

b. Provide a technical review of any application for lab research, contained trial, confined trial, field trial, open field trial, field release, introduction, use, and import and forward recommendations to the NCB for its consideration.

c. Provide technical comments or recommendations to NCB or the government on policy, legal and technical issues of Biosafety as and when requested for.

d. Review all existing bilateral and multilateral projects, and all research that are now being undertaken among universities, research institutes and private organizations.

e. Inspect and certify all laboratories and facilities engaged in GMO research.

f. Coordinate biosafety aspects in public –private sector partnership to ensure safety to ensure safety level in all biotechnological work being carried out under such partnership

g. Approve application for field trials that are renewals or repeat applications from trials previously approved by the NCB.

h. BCC shall take decision safety of the food derieved from GE crops. BCC may invite relevant expert members to take decession on this issue.

i. BCC will meet, at least on a quarterly basis, and may meet additionally as called upon by the Member Secretary or requested by the NCB.

j. Coordinate the activities of Institutional and Field level Biosafety Committees.

k. BCC shall arrange inspection and evaluation of performance of all laboratories engaged in research, development and demonstration of GMOs as and when needed.

l. BCC may co-opt experts related to specific issues regarding Biosafety as and when required.

BCC will meet, at least on a quarterly basis, and may meet additionally to consider urgent matters.

2.2.3 Honorarium

Members shall be entitled per diem as determined by the MOEF.

2.3 Institutional Biosafety Committee (IBC)

All institutions engaged in genetic engineering research shall have an Institutional Biosafety Committee (IBC) to evaluate and monitor the Biosafety aspects of their research. Where an institution intends to become involved in planned field release, members of the IBC should collectively have the range of expertise necessary to supervise and assess the program.

2.3.1. Composition of IBC

Head of the respective Institution/organization shall be appointed as the Chairperson. The committee will be composed of the Chairperson and 4 members from amongst the scientists of the institute to be appointed by the Chairperson according to their expertise in Biosafety of the corresponding modern biotechnological activities. They should be able to evaluate, assess and advise precautionary measures for the modern biotechnological work being conducted at the institution. The IBC represents the most important component in the domain of Biosafety supervising all modern biotechnological work in the Institute.

2.3.2. Powers, Functions and Responsibilities of IBC

2.3.2.1 Powers

The IBC is empowered to enforce all biosafety regulations in the institute/organisation; report infractions to the NCB and to stop a project if its continuation is considered a threat to laboratory personnel, public or environment.

2.3.2.2. Functions

a. Review and recommend application for research, introduction, and import of GMOs/LMOs for considerations by the NCB. Review works conducted by the institutions shall cover:

   i. The containment levels required by the guidelines for the proposed research.

   ii. The facilities, procedures and practices and the training and expertise of personnel assigned to the work.

b. May hold discussions or public consultations, as appropriate, on the objectives of the proposed genetic engineering products/or services.

c. Regular monitoring research project and notify the project chief/proponents/investigator about the results of the review.

d. Review at least once in every year, the work or assessment reports on potential risks being conducted at the institution as well as review laboratory records on regular basis to ensure that requirements of the guidelines are being fulfilled.

e. Formulate and adopt emergency plans covering accidental spills and personnel contamination resulting from lab and fieldwork.

f. Report immediately to the NCB any significant problems with the implementation of the guidelines and any significant research-related accidents or illness.

g. Maintain close liaison with the BCC.

h. Perform such other functions as may be delegated by the NCB.

2.3.2.3. Responsibilities

a. Ensure that laboratory genetic manipulation work within the institution conforms to the regulations of the Biosafety Guidelines.

b. Assess the projects submitted for approval and based on information provided and anticipated risks determine under which category of work the proposal falls and whether to endorse the work proposed.

c. Maintain records of approved project proposals for genetic manipulation work and the committee's assessment

d. Undertake risk assessment in cooperation with research teams if necessary to determine the appropriate containment and biosafety conditions

e. Prepare specific contingency plan after undertaking risk assessments and reviewing project proposal.

f. Monitor the containment features and working conditions within the laboratories, plant glass houses and animal houses of the institute to ensure that various facilities are maintained at the required standard.

2.3.3 Meetings of the IBC

The IBC shall meet every month and hold special meetings to consider urgent matters upon the decision of the Chairperson.

2.3.4 Quorum

Three (3) members including the Chairperson shall constitute a quorum for the transaction of business.

2.3.5 Honorarium:

IBC members may be entitled to such honorarium as may be determined by the institutional head.

2.3.6 Biological Safety Officer (s) (BSO)

Each institution shall designate at least one scientist as Biosafety Officer (BSO). The Biosafety Officer should have considerable experience on biosafety issues and emergency counter measures and is expected to have rigorous training on bio safeguards. It shall be the duty of the BSO to monitor the compliance of the guidelines and rules at the institution level. BSO has to report regularly to the Chairperson of IBC on any matter regarding biosafety applications in the institution.

Honorarium may be paid to the BSO shall be determined by the head of the institute.

2.4 Field level Biosafety Committee (FBC)

A Field level Biosafety Committee (FBC) shall be formed by the NCB to monitor each field trial/release, consisting of a minimum of three (3) members with relevant expertise necessary to monitor and ensure compliance during the field trial/release.

2.4.1 Functions of FBC:

The FBC shall perform the following functions:

a. Monitor the implementation of field trials.
b. Provide recommendations to the trial manager on the implementation of the field trial for compliance with the biosafety guidelines.
c. Report conclusions of monitoring activities to the NCB

2.4.2. Responsibilities

a. Ensure proper implementation of field trials conforms to the regulations of the Biosafety Guidelines.
b. Monitor working conditions that various facilities are maintained at the required standard
c. Report on the above to the NCB.

2.5 Precautionary Measures

The IBC and BSO will ensure that all personnel working on GMOs are well aware of the risks and hazards involved in their work and that the facilities and instruments governing ambient Biosafety are in order. The BSO will adopt a system of reporting laboratory accidents, or other adverse occurrence and the subsequent measures undertaken in dealing with such incidents.

CHAPTER III

GENERAL PROVISIONS ON BIOSAFETY

3.0 RISK ASSESSMENT and RISK MANAGEMENT

3.1 Risk Assessment

3.1.1 Objectives

The objective of risk assessment, under this Guideline, is to identify and evaluate the potential adverse effects of GMOs on the conservation and sustainable use of biological diversity in the likely potential receiving environment, taking also into account risks to human health.

3.1.2 Use of risk assessment

Risk assessment is, inter alia, used by competent national authorities to make informed decisions regarding GMOs. Article 15 of Cartagena Protocol on Biosafety referring to its Annex III gives the basic requirements of risk assessment.

3.1.3 General principles of risk assessment

i. Risk assessment should be carried out in a scientifically sound and transparent manner and can take into account expert advice of, and guidelines developed by, relevant international organizations.

ii. Lack of scientific knowledge or scientific consensus should not necessarily be interpreted as indicating a particular level of risk, an absence of risk, or an acceptable risk.

iii. Risks associated with GMOs/LMOs or products thereof, namely, processed materials that are of genetically/living modified organism origin, containing detectable novel combinations of replicable genetic material obtained through the use of modern biotechnology, should be considered in the context of the risks posed by the non-modified recipients or parental organisms in the likely potential receiving environment.

iv. Risk assessment should be carried out on a case-by-case basis. The required information may vary in nature and level of detail from case to case, depending on the GMO/LMO concerned, its intended use and the likely potential receiving environment.

3.1.4 Methodology of Risk Assessment

The process of risk assessment may on the one hand give rise to a need for further information about specific subjects, which may be identified and requested during the assessment process, while on the other hand information on other subjects may not be relevant in some instances.

Risk assessments for GMOs are conducted on a transgenic event. These assessments are not specific to varieties and are not affected by the breeding history of the GMO. To fulfill its objectives, risk assessment entails, as appropriate, the following steps:

i. An identification of any novel genotypic and phenotypic characteristics associated with the GMO/LMO that may have adverse effects on biological diversity in the likely potential receiving environment, taking also into account risks to human health;

ii. An evaluation of the likelihood of these adverse effects being realized, taking into account the level and kind of exposure of the likely potential receiving environment to the living modified organism;

iii. An evaluation of the consequences should these adverse effects be realized;

iv. An estimation of the overall risk posed by the living modified organism based on the evaluation of the likelihood and consequences of the identified adverse effects being realized;

v. A recommendation as to whether or not the risks are acceptable or manageable, including, where necessary, identification of strategies to manage these risks; and

vi. Where there is uncertainty regarding the level of risk, it may be addressed by requesting further information on the specific issues of concern or by implementing appropriate risk management strategies and/or monitoring the living modified organism in the receiving environment.

3.1.5 Points to consider during Risk Assessment

Depending on the case, risk assessment takes into account the relevant technical and scientific details regarding the characteristics of the following subjects:

i. **Recipient organism or parental organisms:** The biological characteristics of the recipient organism or parental organisms, including information on taxonomic status, common name, origin, centers of origin and centers of genetic diversity, if known, and a description of the habitat where the organisms may persist or proliferate.
ii. **Donor organism or organisms:** Taxonomic status and common name, source, and the relevant biological characteristics of the donor organisms.
iii. **Vector:** Characteristics of the vector, including its identity, if any, and its source or origin, and its host range.
iv. **Insert or inserts and/or characteristics of modification:** Genetic characteristics of the inserted nucleic acid and the function it specifies, and/or characteristics of the modification introduced.
v. **Biological identity and difference between GMOs and Parental organism:** Identity of the GMO and the differences between the biological characteristics of the GMO and those of the recipient organism or parental organisms.
vi. **Detection and identification of the GMO:** Suggested detection and identification methods and their specificity, sensitivity and reliability.
vii. **Information relating to the intended use:** Information relating to the intended use of GMO, including new or changed use compared to the recipient organism or parental organisms; and
viii. **Receiving environment:** Information on the location, geographical, climatic and ecological characteristics, including relevant information on biological diversity and centers of origin of the likely potential receiving environment.

3.1.6 Criteria for Risk Assessment

The criteria for assessing risk should take the following general concepts into consideration:

i. The character of the organism rather than the process used to develop it.
ii. Recognition that risk assessment should be approached with reference to information held in existing data bases, gained from the extensive use of traditionally modified organisms.

iii. The manner in which organisms or their products are to be used (e.g. laboratory, greenhouse, small-scale field trial, industrial production) including any management practices applied, such as provision for containment and waste treatment.

iv. Use of all relevant information, expertise (human capacity) and experience available, in order to allow a judgment of safety or risk.
v. The primary responsibility for the safe use or transfer of organisms GMOs or their products lies with the user. Consequently users should be well informed, competent and well aware of their responsibility.
vi. Consideration and balance of the potential risk with the potential advantages or alternatives and recognition that any approach to implementing guidelines should not impede further development in modern biotechnological research including recombinant DNA.

vii. Consideration of the determined risk potential of donor and recipient organism.

viii. Detailed environmental impact assessment.

3.1.7 Area Specific Risk Assessment Criteria

The assessment of the risk associated with modern biotechnological work can be subdivided into 5 areas depending on how and where GMOs or their products will be used. The specific criteria for each of these areas are given below:

3.1.7.1 Laboratory

i. Good practices for laboratory research should be followed to ensure safety and reproducibility of experiments conducted with GMOs.
ii. Ensure that qualified personnel, appropriate facilities, equipment and materials are available.
iii. Maintenance of records of the qualifications, training, experience and job description for each professional and technical individual.
iv. Ensure that health and safety precautions are applied according to national and/ or international regulations.
v. Risk assessment should be dependent on and be according to the risk group for the GMO being used.

3.1.7.2 Use of GMOs in the field

i. In field testing of GMOs, risks can be minimized or eliminated by confining the introduced organisms to the target environment.
ii. In all cases involving microorganisms, plants and animals the following should be taken into consideration:
    a. Vector host specificity and stability.
    b. Potential for vector "leakage" into unintended hosts in the environment.
    c. Nature and case of possible recombination and spread of such vectors.
iii. Such consideration to be given to the receiving environment e.g. the characteristics of the areas and other organisms that might be affected.
iv. Sound scientific principles should be applied to adequately measure the effects of the introduced organisms on human and the environment.
v. Anticipation that in most cases there will be low environmental risk after modification of an organism by altering, deleting or adding a few genes and its re-introduction into its natural habitat.
vi. Plants with unfamiliar phenotypes should be subject to oversight until their behaviour is predictable and shown to be non-detrimental to the environment.
vii. Ecological uncertainties regarding microorganisms can be addressed scientifically regarding their genetic and phenotypic characteristics.

3.1.7.3 <u>Introduction</u> of foreign GMOs into the environment

i. GMOs that are considered harmless in one region might be potentially harmful in another region with different environmental conditions. Particular stress has to be given to the fact that extreme climatic conditions are prevalent in our country. Therefore adequate field-testing under criteria given above is essential.

ii. Consideration needs to be given to ensure that the introduction of GMO does not interfere with the protection of genetic resources and biological diversity.

Framework for risk assessments for field test and release of GMOs is given in Annex. - 5.

3.1.7.4 Industrial use of GMOs

i. Should consider safe operational procedure such as good occupational hygiene and good microbiological techniques.
ii. Consideration of primary containment procedures in design. For example operation and equipment has to be designed to protect the personnel and the immediate processing facility from exposure to microorganisms.
iii. Consideration of secondary containment procedures such as facilities available to protect the external laboratory or factory environment from exposure to microorganisms.

3.1.7.5 Products intended for release into the market.

i. There should be awareness of the potential allergenicity of any genetically engineered plants or microorganisms and their products. Therefore risk assessment should include scope for evaluation of that potential.

ii. Food safety assessments for novel foods resulting from GMOs should be assessed in the context of international guidelines, national standards and in the context of similar conventional foods.

3.1.8 Procedures and Guidelines for Obtaining Permission in favor of Working with GMOs

Procedures and guidelines may vary according to the biosafety levels and the kind of application of GMOs. Designations of biosafety levels 1-4 have to be given according to description and definitions given in Annex.-1 depending upon type of application e.g. laboratory, field or market.

3.1.8.1 Laboratory

i. Laboratories wanting to work with GMOs need permission from the NCB under the Ministry of Environment and Forests, Government of the People's Republic of Bangladesh. Such permission will be given according to the biosafety level of the organisms to be experimented with. Permission to work for required biosafety level will be asked through the respective IBC and designated according to tables (or organisms) specified in the Annex-1 and Annex -4. Once applications are made by a laboratory, permission needs to be given within 90 days. After permission is given, the specific laboratories will be registered accordingly (with respect to biosafety level) by the member secretary, NCB.
ii. Permission for each specific import of microorganisms for laboratory work at all levels of safety will not be required. NCB will only need to be informed about import of BL-2, BL-3 or BL-4 microorganisms. If required, the NCB will inform customs at the port of entry about permission to import GMOs.

iii. The written application for permission to work with GMOs shall respond to all items listed in items 3.1.9.1-3.1.9.4.
iv. Laboratories permitted to work with GMOs/LMOs have to follow the principles of Good Laboratory Practice (GLP) (Annex.-2).

v. All laboratories have to follow the rules and regulations covered by the Ministry of Commerce during the import of any material related to biotechnology.

Application format for project proposal for laboratory work should include the following information in addition to the general information.

Comment [u3]: The application format should be developed and included as an annex.

a. Title of the Project
b. Name address of the Chief Investigator
c. Objectives of the Project
    i. Overall Objective
    ii. Specific Objective
d. Intended date of commencement
e. Intended date of completion
f. Intended Classification BL1, BL2, BL3 etc
g. Any special precautions to be adopted
h. Details of the biological system to be used (These will give vivid description of the donor, host characteristics, vector, viability etc.)
i. History of prior work with the system being used.
j. Personnel involved in the work and their background
k. Laboratory facilities available
l. Additional Information, if any

In addition the Project Proposal has to include supplementary information for work with
    i. Plants
    ii. Animals
    iii. Fish and aquatic
    iv. Microorganisms

3.1.8.2. Field-release

i. Laboratories/companies/organizations seeking field test and release GMOs or use such organisms for large scale production will need to apply for a separate permit for each individual organism from NCB through the IBC.

ii. Application for a permit of proposed release of GMOs into the environment duly endorsed by the IBC should be submitted to the NCB 90 days in advance of the planned release. The NCB will provide a decision within 90 days, provided the application is complete. The written application shall respond to all items listed in the application procedure provided in items 3.1.9.1-3.1.9.2.

iii. NCB will grant a permit if warranted. If an application is denied, appropriate explanation will be given.

iv. All persons who are granted permit to release GMOs or use such organisms are required to submit periodic reports to the IBC and maintain concept of Good Industrial Large Scale Practice (GILSP) for large scale production (Annexure-3).

v. The NCB, FBC and the IBC will be responsible for monitoring the progress of the work and immediately report any significant outcome to the IBC for remedial action.

vi. Laboratories/companies/organizations permitted to field release GMOs have to maintain detailed records of experiments designed for the purpose of field release.

vii. If any GMOs intended for field release needs to be imported, the NCB will issue an import permit for each individual event and inform the relevant authorities at the port of entry. The import permit will be separate from the permit to field release individual events.

viii. NCB through IBC has to ensure that transgenic organisms imported from outside be grown in a clearly marked, confined space specifically prepared for this purpose; entry to such confined space will be restricted to personnel trained in biosafety measures.

ix. NCB through IBC has to ensure that a reproductive isolation is maintained for GMO plants under testing in confined conditions. Methods for accomplishing this can be found in 3.2.1.1.

x. IBC will ensure that the guideline in Annex.-5 is followed for permission of micro-organism release to be given by the NCB.

xi. For animals, reproduction isolation has to be monitored and animals confined until proven safe to be released into the environment.

~~xiii.~~xii. For GM fish and aquatic, propagation or breeding must be carried out in contained environment and proper safety for environment and human health need to be ensured before their release into open water ecosystems.

Application format for *permission to undertake field release of GMOs* should include the following information in addition to the general information.
(As a prescribed format)

a. Title of the project
b. Name address of the Chief Investigator
c. Objectives of the Project
    i. .Overall Objective
    ii. Specific Objective
d. Intended date of commencement
e. Intended date of completion
f. Location of release with area to be covered
g. Time of release (Date)
h. Expected date of completion of release with area to be covered
i. Information on similar release elsewhere with adverse effect observed (if any)
j. Experimental details with quantity of materials to be released (number, weight, size etc)
k. Is future field release of the same material expected? If yes,
l. Amount time location and period of release
m. What is the intended output of the field release?
n. Precautionary measures to be taken as per Biosafety Guidelines in case of adverse situation,
o. Additional information, if any.

3.1.8.3. **Release into the market**

i. Application for a permit for import and release of individual product has to be submitted to the NCB 90 days in advance of the planned release. The application should respond to all items listed in the application procedure mentioned in items 3.1.9.1-3.1.9.10.

ii. NCB will grant a permit if warranted. If an application is denied/appropriate explanation will be provided

iii. All organization/companies that are granted permit to release products produced from GMOs/LMOs into the market are required to keep records and submit periodic reports to NCB regarding outcome of release or any allergic or adverse effect of released product.

iv. All organizations/companies seeking a permit for release of products from GMOs/LMOs should supply information about the safety of the product and describe any known effects of product after release in markets in the country of origin or other countries.

Application format for obtaining permission to undertake Commercial applications of GMOs should include the following information in addition to the general information. (This application can be submitted only when the field trial of the GMO has been successfully completed without any adverse effect)

a) Summary of field trial data as follows

i. Duration of field test
ii. Location of field test
iii. Scale of field test
iv. Detailed methodology of field test
v. Results obtained
vi. Conclusion

b) Rationale for development of the organism for commercialization
c) Details of specific modifications and for what purpose it was done
d) Environmental Consequences i.e. advantages and disadvantages; economic and social benefits; possible side effects; vertical/horizontal transfer of genes; affects on other related organisms etc.
e) Citations

3.1.9 Application procedure for permission to import, introduce, field trial or release GMOs or their products.

A permit will be issued based on the specific information indicated below:

3.1.9.1 Responsible person or persons involved

i. Name, title, address, telephone number and signature (Head of institute/organization)
ii. Name, address and telephone number of the person(s) who developed and/or supplied the regulated material.

3.1.9.2 Material(s) to be introduced

i. Quantity of the regulated material(s) to be introduced and proposed schedule and number of introductions.
ii. All scientific and common designations necessary to identify the regulated materials.
iii. Country and locality where the regulated material was collected, developed and produced.
iv. Known potential to cause an epidemic (survival and reproductive rates, dispersal, etc.).
v. Classification of regulated material according to Biosafety levels.
vi. Known potential hosts or alternative hosts.
vii. Known ability to evolve.
viii. Known mode of spread and conditions for epidemic.
ix. History of epidemics.

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3.1.9.3 Characteristics of Genetically Modified Microorganisms

i. Nomenclature and detailed characteristics of donor, recipient and vector organisms.
ii. A detailed description of the molecular biology of the systems (e.g., donor-recipient-vector) that is or will be used to produce the regulated materials.

iii. A description of the anticipated or actual expression of the altered genetic material in the regulated materials; an explanation of how that expression differs from the expression in the non-modified parental organism such as morphological or structural characteristics, physiological activities and processes, number of copies inserted in the genetic material; the physical state of this material inside the recipient organism (integrated or extra-chromosomal), products and secretions and growth characteristics.

iv. A detailed description of the processes, procedures and safeguards that have been used or will be used in the country of origin and in Bangladesh to prevent contamination, release and dissemination in the production of the donor organism, recipient organism, vector or vector agent, regulated materials and a constituent of each regulated material which is a product.

3.1.9.4 Additional requirement for field release/commercialization.

i. A detailed description of the uses and the purpose for introducing the regulated material, including a detailed description of the proposed experimental and/or production design.
ii. History of similar introductions.
iii. A description of transfer of the regulated material (e.g., common carrier, baggage or hand-carried).
iv. A detailed description of the intended destination (including final and all intermediate destinations), and/or distribution of the regulated material (e.g., greenhouse, laboratory, or growth chamber location, field trial location, pilot project location, production, propagation and manufacture location, proposed sale and distribution location).
v. A detailed description of the proposed procedures, processes and safeguards that will be used to prevent escape and dissemination of the regulated material in each of the intended destinations.
vi. A detailed description of any biological materials (e.g., culture medium or host material) accompanying the regulated material during movement.
vii. A detailed description of the proposed method of final disposition of the regulated material.
viii. Supervision of planned release.
ix. Contingency plans to cope with extreme conditions (e.g. typhoon).
x. Consequences of the organism remaining in the environment beyond the planned periods.
xi. Methods used (and their sensitivities) to monitor the planned release.
xii. Methods used (and their efficiency) to control or eliminate the organism from the site and the surrounding environment, should circumstances so dictate.
xiii. Identification of the person who will undertake the release of the product in the field.
xiv. Other information :
-Information on the handler (not the broker)
-Knowledge of the handler
xv. On-farm release of GMOs/LMOs: On-farm environmental release procedure of GMOs/LMOs should be developed in association and consultation with the Department of Environment. Advance information/notification should be given to the farmers/community of the test site of GMOs/LMOs.

3.1.9.5 Assessment of subsequent behavior of GMO after field release

Subsequent behavior of GMOs/LMOs in the ecosystem after field release will be monitored by NCB according to guidelines covered in items 1.9.4 and 1.9.5. The time scale for monitoring of organism after field release will be determined by NCB according to risk level of organism being introduced.

3.1.10 Issue of Permit

Issue of permit shall be executed in accordance with the following procedural steps:

i) Permit will be issued according to guidelines specified for laboratory, field or market requirements as mentioned in items 3.1.8 (A), (B) and (C).

ii) Any person whose application has been denied or whose permit has been withdrawn may appeal in writing to the appropriate authorities within 30 days of receipt of the written notice. The appeal should clearly state all the facts and reasons to show that the permit was wrongfully withdrawn or denied. All appeals including application documents shall be referred back to NCB for final comments and suggestions.
iii) Four copies of the permit will be issued. One copy will be retained by the applicant. The other three can be presented at a) port of entry, b) exporting organization, c) collector of customs if required.
iv) A person who is issued a permit should comply with conditions specified in it. Non-compliance with the conditions shall be the ground for revocation of the permit. It will remain revoked until such time that the specified conditions are fully complied with.

3.2 RISK MANAGEMENT

Economic theory suggests a tradeoff between risk and returns, i.e. people who accept higher risk should expect higher returns assuming there are no other alternatives with equal returns less risky. Selecting the appropriate risk-return tradeoff is a critical management decision. Managers have a variety of mechanisms for managing risk. The best method(s) of managing risk depends upon the nature of the risk involved. Four general procedures for managing risk are:
(i) Avoidance, (ii) Reduction, (iii) Assumption/Retention, and (iv) Transfer.

The authority or the institute, who is importing a GMO or any products thereof, for contained use, field trial or introduction, shall vigorously follow the standard procedures of risk management in all the steps. The authority should ensure that there is compliance with the safety conditions that are developed from the results of the risk assessment and management study for a particular GMO. It should include appropriate control procedure and procedures for terminating the experiment and disposing of wastes.

i. Study of risk assessment and management for any particular GMO should be carried out by competent bodies. Multidisciplinary team of relevant expertise should be involved in formulating the risk assessment and risk management plan report. In case of necessity expatriate/foreign expert can be included in the study team. Country of origin and or exporter of GMO shall be requested by the importer to prepare and submit a risk assessment and management plan for a particular GMO. This report should be prepared taken the environmental aspects of Bangladesh into consideration.
ii. Case-by-case evaluation should be the rule based on the promise that the risk of a particular application cannot be determined theoretically, but only empirically.

3.2.1 General Precautions

Comment [AR4]: This implies that these precautions are always required for any GMOs, including those subject to a risk assessment and found not to present any risk to the environment. This is not what you want.

Orientation with appropriate information and training is most important thing to be provided to those involved in handling the GMOs/LMOs. Monitoring procedures are applied in such a way that appropriate measures can be taken in case of unexpected effects during or after the import, contained use, field-trial and release of GMOs. Controlling dissemination of the released organisms and/or gene flow from the released organisms and controlling access to the released site are two fundamental procedures followed in risk management. The general methods for managing risk in handling of various GMOs in different sectors are described below.

These precautions may be waived following a risk assessment indicating that they are unnecessary to prevent harm to the conservation and sustainable use of biodiversity or harm to human and animal health:

3.2.1.1 For plants:

Applying reproductive isolation by, for example:

- spatial separation

- temporal separation, use of plants that will flower either earlier or later than plants of nearby reproductively compatible species
- biological prevention of flowering e.g. by omitting vernalization
- removal of the male or female reproductive structures
- bagging of flowers
- making use of sterility
- controlling the persistence or dispersal of reproductive structures such as propagules or seeds
- destroying volunteer plants after harvest; control of volunteers may be necessary during longer periods, depending on the species

3.2.1.2 **For animals:**

- Confining by appropriate means such as fences, filters, islands, ponds
- Applying reproductive isolation by using sterile animals
- Isolation from feral animals of the same species
- Controlling the persistence or dispersal of reproductive structures such as larvae or eggs

3.2.1.3 For Fish and aquatic:

- Stocking in aquaria, cemented cisterns, ponds and impoundments;
- Applying reproductive isolation by using genetically sterile or triploid fish
- Isolation from feral fish from same species/strains

3.2.1.4 **For microorganisms:**

- Using organisms with impaired ability to grow or persist in the environment
- Minimizing gene transfer by-
  - using organisms that do not contain known transposable genetic elements or known oncogenic genes,
  - ensuring that introduced traits are stably located on the chromosome,
  - using cell line that should be less potentially carcinogenic and
  - using product (virus) that should not carry oncogenic gene

3.2.2 Procedures regarding Movement (Trans-boundary/in-country) and direct use as food or feed, or for processing of GMOs

3.2.2.1. General Principles

i. No GMOs shall be imported and introduced (and moved/transported) unless specified guidelines on packaging and container requirement including marking and identification requirements are fully developed/complied with.
ii. Any movement of regulated material shall be accompanied by relevant permit issued by NCB.
iii. All movements of hazardous organisms of GMOs/LMOs shall include an advanced agreement between the participating countries, laboratories and the IBC or BSO.
iv. All the guidelines for movement shall be applied in the domestic transport within and between institutions.

3.2.2.2. Procedure regarding trans-boundary movement of GMOs

i. An advance informed agreement (AIA) procedure shall be applied by the government prior to the first intentional trans-boundary movement of GMOs for intentional introduction into the environment of the country.
ii. Every country of export shall notify, or require its exporter to ensure notification to, in writing, the competent national authority as may be designated by the government prior to the intentional trans-boundary movement of GMOs/LMOs. The notification shall contain, at a minimum, the information specified in Annex.-6.
iii. The country of export shall ensure that there is a legal requirement for the accuracy of information provided by the exporter.

iv. The competent national authority shall acknowledge receipt of the notification, in writing, to the notifier within 90 (ninety) days of its receipt.

v. The acknowledgement shall state:

a. The date of receipt of the notification;
b. Whether the notification, prima facie, contains the information required;
c. Whether to proceed according to the domestic regulatory framework of the country of import or according to the procedure specified in Article 10 of the Cartagena Protocol.

vi. A failure by the designated authority to acknowledge receipt of a notification shall not imply its consent to an intentional trans-boundary movement.

vii. The designated authority shall, within 90 days informs the notifier, in writing, whether the intentional trans-boundary movement may proceed.

viii. A failure by the designated authority to communicate its decision within two hundred and seventy days of the date of receipt of the notification shall not imply its consent to an intentional trans-boundary movement.

ix. Lack of scientific certainty due to insufficient relevant scientific information and knowledge regarding the extent of the potential adverse effects of a GMO on the conservation and sustainable use of biological diversity in the country, taking also risks to human health into account, shall not prevent the country from taking a decision, as appropriate, with regard to the import of the living modified organism in question, in order to avoid or minimize such potential adverse effects.

3.2.2.3. Safety Procedure regarding direct use of GMOs as food or feed, or for processing

i. While taking a decision on the import of GMOs intended for direct use as food or feed, or for processing, the provisions laid down in Article 11 of the Cartagena Protocol shall be followed.
ii. Lack of scientific certainty due to insufficient relevant scientific information and knowledge regarding the extent of the potential adverse effects of a GMO on the conservation and sustainable use of biological diversity taking also into account risks to human health, shall not prevent the country from taking a decision, as appropriate, with regard to the import of the GMO/LMO intended for direct use as food or feed or for processing, in order to avoid or minimize such potential adverse effects.
iii. While taking a decision on the import of GMOs/LMOs intended for direct use as food or feed, or for processing, the information required in Annex.-7 must be made available.
iv. For import of all GM food products, directly consumable/drinkable or be consumed/drunk after processing, the importer shall require to submit along with other shipping documents a cautionary certificate from the government of the exporting country or from appropriate approving agency to the effect that the item is “fit for human consumption”, that it does not contain harmful ingredients” or that “it is free from all kinds of harmful germs”. Such a certificate shall mention the ‘‘age group for which the item is eligible for consumption’’.

3.2.2.4.. Procedure regarding handling, transport, packaging and identification of GMOs/LMOs

i. In order to avoid adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health, the government shall require that GMOs/LMOs organisms that are subject to intentional trans-boundary movement are handled, packaged and transported under conditions of safety, taking into consideration relevant international rules and standards.
ii. Bangladesh shall take measures to require that documentation accompanying:

(a) GMOs/LMOs that are intended for direct use as food or feed, or for processing, clearly identifies that they "may contain" GMOs/LMOs and are not intended for intentional introduction into the environment, as well as a contact point for further information;

(b) GMOs that are destined for contained use clearly identifies them as GMOs; and specifies any requirements for the safe handling, storage, transport and use, the contact point for further information, including the name and address of the individual and institution to whom the GMOs are consigned; and

(c) GMOs that are intended for intentional introduction into the environment of the country and any other GMOs, clearly identifies them as such; specifies the identity and relevant traits and/or characteristics, any requirements for the safe handling, storage, transport and use, the contact point for further information and, as appropriate, the name and address of the importer and exporter; and contains a declaration that the movement is in conformity with these requirements.

3.3. Sanctions against violation of Biosafety Guidelines

In addition to the revocation of the project approval, any violation of the provisions of this guideline or the concealment or withholding by the proponent of any information necessary to evaluate risks to human health or the environment shall be penalized by the concerned ministry by stopping the work immediately and forfeiting the government grants/funds.

Further, any incentives that may have been granted the proponent or institution for contributing to advanced scientific or technological research and development will be withheld. These penalties are exclusive of any other penalties tenable by existing Biosafety Rules of Bangladesh.

The NCB will inform the government (MOEF) on all issues pertaining to violations of these guidelines by any organization. The Ministry will, if necessary issue public statement on any such violation to caution other organizations.

CHAPTER IV

PHYSICO-CHEMICAL AND BIOLOGICAL CONTAINMENTS: PROCEDURES AND FACILITIES

4.0 Measures for Containment

Since research and development in modern biotechnology may have adverse impact on biodiversity and human health, precautionary measure is a prerequisite for each and every laboratory in the country. Therefore, measures should be taken to limit the interaction of the regulated organisms with the environment or with human being. For this the procedures for physico-chemical and biological containments have been given in details in this chapter.

4.1. Physical containment

4.1.1. Standard practices and training

The first principle of containment is strict adherence to good Biosafety practices. Consequently, all personnel directly or indirectly involved in experiments on rDNAs and potentially harmful microorganisms, cell lines must receive adequate instruction. This shall include, at the least, instructions in aseptic techniques and in the biology of the organisms used in the experiments so that potential biohazards can be understood and appreciated.

Education and training of personnel is the most important element to maintain Biosafety at all levels, since human error and poor laboratory practice may compromise even the most sophisticated laboratory safeguards and equipment designed to protect workers and the environment. However, the objectives of occupational safety and avoidance of adverse environmental effects can be hardly achieved by mere routine information on sources of danger. Laboratory and plant personnel must be motivated to understand safety at the workplace as an obvious necessity and an essential feature of good professional practice and successful work. Moreover, the best available guideline cannot anticipate every possible situation. Thus education, motivation and good judgment are key essential to safety in biotechnology.

There must be a continuous feedback between the results of safety reviews and both the risk assessment and the safety measures. As a result of this interaction, especially in the case of a new process for which little or no previous experience exists, safety measures may be reduced after some time. More importantly appropriate scrutiny is necessary in order to be able to react immediately in any case of recognized and perhaps unexpected hazard.

Any group working with regulated materials should have an emergency plan which describes the procedures to be followed if an accident contaminates personnel or the environment. Everyone should know about this plan. Physical Containment Level I (BL 1) must ensure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan. If a group is working with a known pathogen for which there is an effective vaccine, such vaccine should be made available to all workers. Where serological monitoring is clearly appropriate, it should be provided.

4.1.2. Physical containment levels

The objective of physical containment is to confine harmful organisms and those containing rDNA molecules and thus reduce the risk of exposure of the laboratory worker and persons outside of the laboratory and the environment to these harmful organisms. The primary means of physical containment equipment. Special laboratory design provides secondary means of protection against accidental release or organisms outside the laboratory or to the environment. Special laboratory design is used primarily in facilities wherein experiments of moderate to high potential hazards are performed.

Combinations of laboratory practices, containment equipment and special laboratory design can be made to achieve different levels of physical containment. Four levels of physical containment, which are designated as BL1, BL2, BL3 and BL4 are described in succeeding paragraphs. It should be emphasized that the descriptions and assignments of physical containment detailed below are based on existing approaches to pathogenic organisms.

It is recognized that several different combinations of laboratory practices, containment, equipment and special laboratory design may be appropriate for containment of specific research activities. The Guidelines, therefore, allow alternative selections of primary containment equipments within facilities that have been designed to provide BL3 and BL4 levels of physical containment. The selection of alternative methods of primary containment depends however, on the level of biological containment, provided by the host-vector system used in the experiment. Consideration will also be given by NCB to other combinations that achieve an equivalent level of containment.

4.1.3. Biosafety level 1 (BL1)

BL1 is suitable for work involving agents of no known or minimal potential hazard to laboratory personnel and environment. The laboratory is not separated from the general traffic patterns in the building. Work is generally conducted on open bench tops. Special containment equipments is not required or generally used. Laboratory personnel work in the laboratory and are supervised by a scientist with general training in microbiology or a related science.

4.1.3.1. Procedures to maintain BL1

i. When experiments are in progress, access to the laboratory is limited or restricted at the discretion of the laboratory director.

ii. Work surfaces are decontaminated once a day and after any spill of viable material.

iii. All contaminated liquid or solid wastes are decontaminated before disposal.

iv. Mechanical pipetting devices are used; mouth pipetting is prohibited.

v. Eating, drinking, smoking and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for that purpose only.

vi. Persons wash their hands after they handle materials involving organisms containing rDNA molecules and animals and before leaving the laboratory.

vii. All procedures are performed carefully to minimize the creation of aerosols.

viii. Laboratory personnel wear laboratory coats, gowns or uniforms to prevent contamination or soiling of street clothes.

ix. Contaminated materials that are to be decontaminated at a site away from the laboratory should be placed in a durable, leak-proof container, which is closed before being removed from the laboratory.

4.1.3.2. Containment equipment:

Special containment is generally not required for manipulations of agents assigned to BL1

4.1.3.3. Laboratory Facilities for BL1

i. The laboratory is designed in such a way that it can be cleaned easily.

ii. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents and moderate heat.

iii. Laboratory furniture is sturdy. Space between benches, cabinets and equipment are accessible for cleaning.

iv. Each laboratory contains a sink for hand washing.

v. Laboratory windows are fitted with fly screens;

vi. Each laboratory contains proper fume hood and acid dispense facilities.

4.1.4. Biosafety Level 2 (BL2)

BL2 is similar to BL1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that (1) laboratory personnel are directed by experienced scientists, (2) access to the laboratory is limited when work is being conducted, and (3) certain procedures (in which infectious aerosols are created) are conducted in biological safety cabinets or other physical containment equipments.

4.1.4.1. Procedures to maintain BL2

All procedures under BL1 are applicable. In addition, the following procedures should be followed:

i. Experiments of lesser biohazard potential can be carried out concurrently in carefully demarcated areas of the same laboratory.

ii. The Laboratory director limits access to the laboratory. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

iii. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazard and who meet specific entry requirements (e.g., immunization) can enter the laboratory or animal rooms.

iv. When the organisms containing rDNA molecules in use in the laboratory require special provisions for entry (e.g., vaccination), a hazard warning sign incorporating the universal biohazard symbol (see Annex.-8) is posted on the access door to the laboratory work area. The hazard warning sign identifies the agent, lists the name and telephone number of the laboratory director or other responsible persons, and indicates the special requirements for entering the laboratory. An insect and rodent control program is in effect as certified by a licensed pest control operator.

v. Laboratory coats, gowns, smocks, or uniforms are worn by personnel while in the laboratory. While entering the laboratory, personnel should change the shoes and wear shoes specified for the laboratory. Before personnel leave the laboratory for non-laboratory areas (e.g., cafeteria, library, administrative offices etc.), this protective clothing is removed and left in the laboratory or is covered with a clean coat not used in the laboratory.

vi. Animals not involved in the work being performed are not permitted in the laboratory.

vii. Special care is taken to avoid skin contamination with organisms containing rDNA molecules; gloves should be worn when handling experimental animals and when skin contact with the agent is unavoidable.

viii. All wastes from laboratories and animal rooms are appropriately decontaminated according to acceptable minimum standards for proper disposal.

ix. Hypodermic needles and syringe are used only for parental injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of fluids containing organisms that have rDNA molecules. Extreme caution should be observed when handling needles and syringes to avoid auto-inoculation and generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture - resistant container and decontaminated, preferably by autoclaving, before discard or re-use.

x. Spills and accidents that result in over-exposures to organisms containing rDNA molecules are immediately reported to the laboratory director and the IBC. Medical evaluation, surveillance and treatment are provided as appropriate and written records are maintained.

xi. When appropriate, considering the agent (s) handle, baseline serum samples for laboratory and other personnel at-risk are collected and stored. Additional serum specimens may be collected periodically, depending on the agent handles or the function of the facility.

xii. A Biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read instructions on practices and procedures and to follow them.

4.1.4.2. Containment equipment required in BL2

i. Biological safety cabinets (see Section 4.4, Class I or II) or other appropriate personal protective or physical containment devices are used whenever necessary.

ii. Proper containment is applied when procedures with a high potential for creating aerosols are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of materials whose internal pressures may be different from ambient pressures, inoculating animals and harvesting infected tissues from animals or eggs.

iii. Proper containment is applied when high concentrations or large volumes of organisms containing rDNA molecules are used. Such materials may be centrifuged in the open laboratory if sealed heads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

4.1.4.3. Laboratory Facilities required in BL2:

All facilities under BL1 as mentioned in 4.1.3.3. are applicable. In addition the following facility should be present.

i. An autoclave for decontaminating laboratory wastes is available.

4.1.5. Biosafety Level 3 (BL3)

BL3 is applicable to clinical diagnosis, teaching, research or production facilities where work is done with indigenous or exotic agents that may cause serious or potentially lethal diseases as a result of exposure by inhalation. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents and are supervised by competent scientists who are experienced in working with these agents. All procedures involving manipulation of infectious material are conducted within biological safety cabinets or other physical containment devices. Personnel wear appropriate personal protective clothing and devices. The laboratory has special engineering and design features. It is recognized, however, that existing facilities may not have all the facility safeguards recommended for BL3 (e.g., access zone sealed penetrations and directional airflow, etc.). In such cases, the proponent must show proof of access to BL3 facilities. Under these circumstances, acceptable safety may be achieved for routine or repetitive operations (e.g., diagnostic procedures involving the propagation of agent for identification, typing and susceptibility testing) in laboratories where facility features satisfy BL2 recommendations, provided the recommended "Standard Microbiological Practices", "Special Practices" and "Containment Equipment" for BL3 are rigorously followed. The decision to implement this modification of BL3 recommendations should be made only by the laboratory director.

4.1.5.1. Procedures applicable for BL3:

All procedures under BL1 (4.1.3.1) and BL2 (4.1.4.1) are applicable. In addition the following procedures have to be followed for BL3.

i. All contaminated liquid or solid wastes are decontaminated before disposal.
ii. Persons under 18 years of age are not allowed to enter the laboratory.

iii. If experiments involving other organisms that require lower levels of containment are to be conducted in the same laboratory concurrently with work requiring BL3 level physical containment, such experiments shall be conducted in accordance with all BL3 level practices.
iv. Laboratory doors are kept closed when experiments are in progress.
v. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable, leak proof container, which is closed before being removed from the laboratory.
vi. The laboratory director controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. The director has the final responsibility of assessing each circumstance and determining who may enter or work in the laboratory.
vii. The laboratory director establishes policies and procedures whereby only persons who have been adduced of the potential biohazard who meet any specific entry requirements (e.g., immunization) and who comply with all entry and exit procedures enter the laboratory of animal rooms.
viii. When organisms containing rDNA molecules or experimental animals are present in the laboratory or containment module, a hazard warning sign incorporating the universal biohazard symbol is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the laboratory director or other responsible persons and indicates any special requirements for entering the laboratory, such as the need for immunization, respirators or other personal protective measures.
ix. All activities involving organisms containing rDNA molecules are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench.
x. The work surfaces of biological safety cabinets and other containment equipment are decontaminated when work with organisms containing rDNA molecules is finished. Plastic-backed paper toweling used on non-perforated work surfaces within biological safety cabinets facilitates cleaning up. An insect and rodent program is in effect as certified by a licensed pest control operator.
xi. Laboratory clothing that protects street clothing (e.g., solid front or wrap-around gowns, scrub suits, cover-alls) is worn in the laboratory. Laboratory clothing is not worn outside the laboratory and is decontaminated before being laundered.
xii. Special care is taken to avoid skin contact with contaminated materials; gloves should be worn when handling infected animals and when skin contact with infectious materials is unavoidable.
xiii. Molded surgical masks or respirators are worn in rooms containing experimental animals.
xiv. Animals and plants not related to the work being conducted are not permitted in the laboratory.
xv. Laboratory animals held in a BL3 area are housed in partial-containment caging systems, such as Horsfall units, open cages placed in ventilated enclosures, solid-wall and bottom cages covered by filter bonnets or solid-wall and bottom cages placed on holding racks equipped with ultraviolet radiation lamps and reflectors.

(NOTE: Conventional caging systems may be used provided that all personnel wear appropriate personal protective devices. These shall include at a minimum, wrap-around gowns, head covers, gloves, shoe covers, and respirators. All personnel shall shower on exit from areas where these devices are required.)

xvi. All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.
xvii. Vacuum lines are protected with High Efficiency Particulate Air (HEPA) filters and liquid disinfectant traps.
xvii. Baseline serum samples for all laboratory and other personnel at-risk should be collected and stored for reference purposes. Additional serum specimens may be collected periodically depending on the agents handled or function of the laboratory.
xviii. Alternative selection of containment equipment is possible. Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified can be conducted in the BL3 laboratory, using containment equipment specified for the BL2 level of physical containment. Experimental procedures involving a host-vector system that provides a one-step lower level of biological containment than that specified can be conducted in the BL3 laboratory using containment equipment specified for the BL4 level of physical containment.

4.1.5.2. Containment equipment

Biological safety cabinets (see Section 4, Class I, II, or III) or other appropriate combinations of personal, protective or physical containment devices (e.g., special protective clothing, masks, gloves, respirators, centrifuge, safety cups, sealed centrifuge rotors, and containment caging for animals) are used for all activities with organisms containing rDNA molecules, which pose a threat of aerosal exposure. These include : manipulation of cultures and of clinical or environmental materials which may be a source of aerosols; the aerosol challenge of experimental animals, harvesting infected tissues or fluids from experimental animals and embryonate eggs, and necropsy of experimental animals.

4.1.5.3. Laboratory facilities

i. The laboratory is separated from areas which are open to unrestricted traffic flow within the building. Passage through two sets of doors is the basic requirement for entry into the laboratory from access corridor or other contiguous areas. Physical separation of the high containment laboratory from access corridors or other laboratories or activities may also be provided by a double-door clothes change room (showers may be included), air lock, or other access facility which requires passage through two sets of doors before entering the laboratory.

ii. The interior surfaces of walls, floors and ceilings are water resistant so that they can be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate decontamination of the area.

iii. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents and moderate heat.

iv. Laboratory furniture is sturdy and spaces between benches, cabinets and equipment are accessible for cleaning.

v. Each laboratory contains a sink for hand washing. The sink may be operated by foot, by elbow or automatically and is located near the laboratory exit door.

vi. Windows in the laboratory are closed and sealed.

vii. Access doors to the laboratory or containment module are self-closing.

viii. An autoclave for decontaminating laboratory waste is available, preferably within the laboratory.

ix. A ducted exhaust air ventilation system is provided. This system creates directional airflow that draws air into the laboratory through the entry area. The exhaust air is not recalculated to any other area of the building, is discharged to the outside, and is dispersed away form the occupied areas and air intakes. Personnel must verify that the direction of the airflow (into the laboratory) is proper. The exhaust air from the laboratory room should be filtered before it is discharged to the outside to be sure it is not contaminated.

x. The HEPA-filtered exhaust air from Class I or Class II biological safety cabinets may be recalculated within the laboratory if the cabinet is tested and certified at least every 12 months. If the HEPA-filtered exhaust air from Class I or II biological safety cabinets is to be discharged to the outside through the building exhaust air system, it should be connected in a manner [e.g., thimble unit connection] that avoids any interference with the air balance of the cabinets or building's exhaust system.

4.1.6. Biosafety Level 4 (BL4)

Work with dangerous and toxic agents which pose a high individual risk of life, threatening disease, members of the laboratory staff are to receive specific training in handling infectious agents. They should be supervised by competent scientists who are trained and experienced in working with specific agents. BL4 provides the most stringent containment conditions. All requirements listed in BL3 are applicable to BL4. Standard microbiological practices should be followed.

4.1.6.1. Procedures:

All procedures under BL1, BL2 and BL3 are applicable. In addition, the following procedures need to be followed for BL4.

i. Biological materials to be removed from the Class III cabinets or from the maximum containment laboratory in a viable or intact state are transferred to a non-breakable, sealed primary container, and then enclosed in a non-breakable, sealed secondary container, which is removed from the facility through a disinfectant dunk tank, fumigation chamber or an air lock designed for this purpose.

ii. No material, except biological materials that are to remain in a viable or intact state, is removed from the maximum containment laboratory unless it has been autoclaved or decontaminated. Equipment or material that might be damaged by high temperatures or temperatures or steam is decontaminated by gaseous or vapor methods in an air lock or chamber designed for that purpose.

iii. Only persons whose presence are required for program or support purposes in the facility or individual laboratory rooms are authorized to enter. The laboratory director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. Access to the facility is limited by means of secure, locked doors. Accessibility is managed by the laboratory director, biohazard control officer or other persons responsible for the physical security of the facility. Before entering, persons are advised of the potential biohazards and instructed on appropriate safeguards to ensure their safety. Authorized persons comply with the instructions and all other applicable entry and exit procedures. A log book signed by all personnel indicates the date and time of each entry and exit, practical and effective protocols for emergency situations are established.

iv. Personnel enter and leave the facility only through the clothing change and shower rooms. Personnel shower every time they leave the facility and use the air locks to enter or leave the laboratory only in an emergency.

v. Street clothing is removed in the outer clothing change room and kept there. Complete laboratory clothing, including undergarments, pants and shirts or jumpsuits, shoes, and gloves are provided and used by all personnel entering the facility. Head covers are provided for personnel who do not wash their hair during the exit shower. When leaving the laboratory and before proceeding into the shower area, personnel remove their laboratory clothing and store it in a locker or hanger in the inner change room.

vi. When materials that have organisms containing rDNA molecules or experimental animals are present in the laboratory or animal rooms, a hazard warning sign incorporating the universal biohazard symbol (Annex.-8) is posted on all access doors. The sign identifies the agent, lists the name of the laboratory director or other responsible persons(s), and indicates any special requirements for entering the area (e.g., the need for immunization or respirators).

vii. Supplies and materials needed in the facility are brought in through the double-door autoclave, fumigation chamber, or airlock which is appropriately decontaminated between each use. After securing the outer doors, personnel within the facility retrieve the materials by opening the interior doors are secured after materials are brought into the facility. An insect and rodent control program is in effect as certified by a licensed pest control operator for all levels.

viii. Materials (e.g., plants, animals and clothing) not related to the experiment being conducted are not permitted in the facility.

ix. Hypodermic needles and syringes are used only for parental injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral part of unit) are used for the injection or aspiration of fluids containing organisms that contain rDNA molecules. Needles should not be bent, sheared, replaced in the needle sheath or guard or removed from the syringe following use. The needle and syringe should be placed in a puncture-resistant container and decontaminated, preferably by autoclaving before discard or re-use. Whenever possible, channels are used instead of sharp needles.

x. A system is set up for reporting laboratory accidents and exposures and employee absenteeism, and for the medical surveillance of potential laboratory-associated illnesses. Written records are prepared and maintained. An essential adjunct to such a reporting surveillance system is the availability of a facility for quarantine, isolation and medical care of personnel with potential or known laboratory-associated illnesses.

xi. Laboratory animals involved in experiments requiring BL4 level physical containment shall be housed either in cages contained in class III cabinets or in partial containment caging systems such as Horsfall units, open cages

placed in ventilated enclosures or solid-wall and bottom cages placed on holding racks equipped with ultraviolet irradiation lamps and reflectors that are located in a specially designed area in which all personnel are required to wear one-piece positive pressure suits.

xii. Alternative selection of containment equipment is possible. Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified can be conducted in the BL4 facility using containment equipment requirements specified for the BL3 level or physical containment.

4.1.6.2. Containment equipment

All procedures within the facility with agents assigned to BL4 are conducted in the Class III biological safety cabinet; or in Class I or II biological safety cabinets used in conjunction with one-piece positive pressure personnel suits ventilated by a life-support system.

4.1.6.3 Laboratory facilities

i. The maximum containment facility consists of either a separate building or a clearly demarcated and isolated zone within a building. Outer and inner change rooms separated by a shower are provided for personnel entering and leaving the facility. A double-door autoclave, fumigation chamber, or ventilated air lock is provided for passage of materials, supplies, or equipment that is not brought into the facility through the change room.

ii. Walls, floors and ceilings of the facility are constructed to form a sealed internal shell that facilitates fumigation and is animal and insect-proof. The internal surfaces of this shell are resistant to liquids and chemicals, thus facilitating cleaning and decontamination of the area. All penetrations in these structures and surfaces are sealed. Any drains in the floors should contain traps filled with a chemical disinfectant of demonstrated efficacy against the target agent, and these are connected directly to the liquid waste decontamination system. Sewer and other ventilation lines contain HEPA filters.

iii. Internal facility appliances, such as light fixtures, air ducts and utility pipes are arranged to minimize the horizontal surface area on which dust can settle.

iv. Bench tops have seamless surfaces that are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

v. Laboratory furniture is made of simple and sturdy material and spaces between benches, cabinets and equipment are accessible for cleaning.

vi. A hand-washing sink operated by foot, by elbow, or automatically is provided near the door of each laboratory room in the facility.

vii. If there is a central vacuum system, it does not serve areas outside the facility. In-line HEPA filters are placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement. Other liquid and gas services to the facility are protected by devices that prevent backflow.

viii. If water fountains are provided, they are foot-operated and are located in the facility corridors outside the laboratory. The water service to the fountain is not connected to the backflow-protected distribution system supplying water to the laboratory areas.

ix. Access doors to the laboratory are self-closing and can be locked. All windows are breakage resistant.

x. A double-door autoclave is provided for decontaminating materials passing out of the facility. The autoclave door, which opens to the area external to the facility, is sealed to the outer wall and automatically controlled so that the outside door can be opened only after the autoclave “sterilization” cycle has been completed.

xi. A pass-through dunk tank, fumigation chamber or an equivalent decontamination method is provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the facility.

xii. Liquid effluents from laboratory sinks, biological safety cabinets, floors and autoclave chambers are decontaminated by heat treatment before being released from the maximum containment facility. Liquid wastes from shower rooms and toilets may be decontaminated with chemical disinfectants or by heat in the liquid waste decontamination system. The procedure used for heat decontamination of liquid wastes is evaluated mechanically and biologically by using a recording thermometer and an indicator microorganism with a defined heat susceptibility pattern. If liquid wastes from the shower room are decontaminated with chemical disinfectants, the chemical used should have demonstrated efficacy against the target or indication microorganisms.

xiii. An individual supply and exhaust air ventilation system is provided. The system maintains pressure differentials and outside of the facility toward areas of highest potential risk within the facility. Manometers are used to sense pressure differentials between adjacent areas maintained at different pressure levels. If a system malfunctions, the manometers sound an alarm. The supply and exhaust airflow is interlocked sound an alarm. The supply and exhaust airflow is interlocked to assure inward (or zero) airflow at all times.

xiv. The exhaust air from the facility is filtered through HEPA filters and discharged to the outside so that it is dispersed away from occupied buildings and air intakes. Within the facility, the filters are located as near the laboratories as practicable in order to reduce the length of potentially contaminated air ducts. The filter chambers are designed to allow in situ decontamination before filters are removed and to facilitate certification testing after they are replaced. Coarse filters and HEPA filters are provided to trap air supplied to the facility in order to increase the lifetime of the exhaust HEPA filters and to protect the air supply system should air pressures become unbalanced in the laboratory.

xv. The treated exhaust air from Class I an II biological safety cabinets can be discharged into the laboratory room environment or outside through the facility air exhaust system. If exhaust air from Class I or II biological safety cabinets is discharged into the laboratory, the cabinets are tested and certified at 6 month intervals. The exhaust air from Class III biological safety cabinets is discharged, without recirculation through two sets of HEPA filters in series, via the facility exhaust air system. If the treated exhaust air from any of these cabinets is discharged to the outside through the facility exhaust air system, the treated exhaust air is connected to this system in a manner [e.g., thimble unit connection] that avoids any interference with the air balance of the cabinets or the facility exhaust air system.

xvi. A specially designed suit area may be provided in the facility. Personnel who enter this area wear a one-piece positive pressure suit that is ventilated by a life-support system. The life-support system includes alarms and emergency backup breathing air tanks. Entry to this area is through an air lock fitted with airtight doors. A chemical shower is provided to decontaminate the surface of the suit before the worker leaves the area. The exhaust air from the suit-area is filtered by two sets of HEPA filters installed in series. A duplicate filtration unit, exhaust fan and an automatically starting emergency power source are provided. The air pressure within the suit area is lower than that in any adjacent area. Emergency lighting and communication systems are provided. All penetrations into the internal shell of the suit area are sealed. A double-door autoclave is provided for decontaminating waste materials to be removed from the suit area.

4.2. Biological containment

In considering biological containment the vector (plasmid, organelle, or virus) for the rDNA and the host (bacteria, plant or animal cell) in which the vector is propagated in the laboratory will be considered together. In any combination of vector and host, the biological containment must be chosen or constructed so that the following types of “escape” are minimized: (a) survival of the vector in its host outside the laboratory and (b) transmission of the vector from the propagation host to other non-laboratory hosts. The following levels of biological containment for host-vector systems (HV) for prokaryotes will be established and specific criteria will depend on the organisms to be used:

i. **HV1.** A host-vector system that requires a moderate level of containment.
Specific systems follow:

EK1: The host is always E. coli K-12 – 102 or a derivative thereof, and the vectors include non-conjugative plasmid (e.g., PSC101, Co (F) or derivatives thereof, and variants of bacteriophage such as lambda. The E. coli K-12 hosts should not contain configuration proficient plasmids, whether autonomous or integrated or generalized transducing phages.

Other HV1 Hosts and vectors shall be, at a minimum comparable in containment to E. coli K-12 with a non-conjugative plasmid or bacteriophage vector. The data to be considered and a mechanism for approval of such HV1 Systems are described in the Annex-1.

ii. **HV2.** These are host vector systems shown to provide a high level of biological containment as demonstrated by data from suitable test performed in the laboratory. Escape of the rDNA either via survival of the organisms or via transmission of rDNA to other organisms should be less than 1/104 under specified conditions. Specific systems are as follows:

EK2. For EK2 host vector systems in which the vector is a plasmid, no more than one in 104 host cells should be able to perpetuate a cloned DNA fragment under the specified non-permissive laboratory conditions designed to represent the natural environment, either by survival of the original host or as a consequence of transmission of the cloned DNA fragment.

For EK2 host vector system in which the vector is a phage, no more than one in 104 phage particles should be able to perpetuate a cloned DNA fragment under the specified non-permissive laboratory conditions designed to represent the natural environment, either by survival of the original host or as consequence of transmission of the cloned DNA fragment.

For EK2 host vector system in which the vector is a phage, no more than one in 104 phage particles should be able to perpetuate a cloned DNA fragment under the specified non-permissive laboratory conditions designed to represent the natural environment either (a) as a prophage (in the inserted or plasmid form) in the laboratory host used for phage propagation or (b) by surviving in natural environment and transferring a cloned DNA fragment to other hosts (or their resident prophages).

4.2.1. Organisms of different safety levels

Organisms of different safety levels with their respective risk groups are provided in Annex.-4.

4.2.2. Certification of host-vector systems

i. Responsibility. HV1 systems other than E. coli K-1 and H2 host-vector systems may not be designated as such until they have been certified by the chairperson of the NCB. Application for certification of a host-vector system should be written and address to:

The Chairperson
National Committee on Biosafety (NCB).

ii. Host-vector systems that are proposed for certification will be reviewed by the NCB. Prior to this, a review of the data on construction, properties and testing of the proposed host-vector system will be made by a working group composed of one or more members of the NCB and other person chosen because of their expertise in evaluating such data. The NCB will then evaluate the report of the working group and any other available information at a regular meeting.
The Chairperson of the NCB is responsible for certification after receiving the advice of the working group. Minor modifications of existing certified host-vector systems, i.e., those of minimal or no consequence to the properties relevant to containment, may be certified by the NCB Chairperson.

ii. When a new host-vector system is certified, NCB sends a notice of the certification to the applicant and to all IBCs and publishes it. Copies of a list of all currently certified host-vector systems may be obtained from NCB at any time.

iv. The NCB may, at any time, rescind the certification of any host-vector system. If certification of host-vector system is rescinded, NCB will instruct investigators to transfer cloned DNA into a different system or use the clones at a higher physical containment level unless the NCB determines that the already constructed clones have adequate biological containment.

v. Certification of a given system does not extend to modifications of either the host or vector component of that system. Such modified systems must be independently certified by the NCB Chairperson, if modifications are minor, it may only be necessary for the investigator to submit data showing that the modifications have either improved or not impaired the major phenotypic traits on which the containment of the system depends. Substantial modifications of a certified system require the submission of complete testing data.

4.2.3. Data to be submitted for certification

i. **HV1 systems other than E. coli K-12:** The following types of data shall be submitted, modified as appropriate for the particular system being considered: (i) a description of the organism and vector, the strain's natural habitat and growth requirements; its physiological properties, particularly those related to its reproduction and survival and the mechanisms by which it exchanges genetic information; the range of organism with which this organism normally exchanges genetic information and what sort of information is exchanged; and any relevant information on its pathogenicity or toxicity; (ii) a description of the history of the particular strains and vectors to be used, including data on any mutations that render this organism less able to survive or transmit genetic information and (iii) a general description of the range of experiments contemplated with emphasis on the need for developing such an HV1 system.

ii. **HV2 Systems:** Investigators planning to request HV2 certification for host-vector systems can obtain instructions from NCB concerning data to be submitted. In general, the following types of data are required: (i) description of construction steps with indication of source, properties, and manner of introduction of genetic traits; (ii) quantitative data on the stability of genetic traits that contribute to the containment of the system; (iii) data on the survival of the host-vector system under non-permissive laboratory conditions designed to represent the relevant natural environment; (iv) data on transmissibility of the vector and/or a cloned DNA fragment under both permissive and non-permissive conditions; (v) data on all other properties of the system which affect containment and utility, including information on yields of phage or plasmid molecules, ease of DNA isolation and ease of transduction or transformation. In some cases, the investigator may be asked to submit data on survival and vector transmissibility from experiments in which the host vector is fed to laboratory animals and human subjects. Such in vivo data may be required to confirm the validity of predicting in vivo survival on the basis of in vitro experiments. Data must be submitted in writing to NCB. A period of 10 to 12 weeks is normally required for review and circulation of the data. Investigators are encouraged to publish their data on the construction, properties and testing of proposed HV2 systems before the system is considered by the NCB and its subcommittee.

4.3. Physical containment for large-scale uses of organisms containing recombinant DNA molecules

This part of the guidelines specifies physical containment facilities for large-scale (for commercial purposes) research or production involving viable organisms containing rDNA molecules. It shall apply to large-scale research or production activities.

All provisions of the Guidelines shall apply to large-scale research or production activities, with the following modifications:

i. The institution shall appoint Biological Safety Officer(s) (BSO) if it engages in large-scale research or production activities involving viable organisms containing rDNA molecules.

ii. The institution shall establish and maintain a health surveillance program for personnel engaged in large-scale research or production activities involving viable organisms containing rDNA molecules, which require BL3 containment at the laboratory scale.

iii. The program shall include pre-assignment and periodic physical and medical examinations; collection, maintenance and analysis of serum specimens for monitoring serologic changes that may result from the employee's work, experience and provisions for investigating any serious, unusual or extended illnesses of employees to determine possible occupational origin.

4.3.1. Selecting physical containment levels

The selection of the physical containment level required for rDNA research or production involving more than 10 liters of culture is based on the containment guidelines established in 5.3.2. For large-scale research or production, three physical containment levels are established: BL1-LS, BL2-LS and BL3-LS.

The BL1-LS level of physical containment level is required for large-scale research or production of viable organisms containing rDNA molecules that require BL2 containment at the laboratory scale.

The BL3-LS level is required for large-scale research or production of viable organisms containing rDNA molecules that require BL4 containment at the laboratory scale. If necessary, these requirements will be established by NCB on an individual basis.

4.3.1.1. BL1-LS Level

i. Cultures of viable organisms containing rDNA molecules shall be handled in a closed system (e.g., closed vessel used for propagating and growing cultures) or other primary containment equipment (e.g., biological safety cabinet containing a centrifuge used to process culture fluids) designed to reduce the potential for escape of viable organisms. Volumes less than 10 liters may be handled outside of a closed system or other primary containment equipment, provided all physical containment requirements specified in 5.3.1 are met.

ii. Culture fluids (except as allowed in 3.1.2.3) shall not be removed from devised system or other primary containment equipment unless the viable organisms containing rDNA molecules have been inactivated by a validation inactivation procedure. A validation inactivation procedure is one that has been demonstrated to be effective using the organism that will serve as the host for propagating the rDNA molecules

iii. Sample collection from a closed system and transferring culture fluids from closed system to another shall be done in a manner which minimizes the release of aerosols or contamination of exposed surfaces.

iv. Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters that have efficiencies equivalent to HEPA filters or by other equivalent procedures (e.g., incineration) to minimize the release of viable organisms containing rDNA molecules.

v. A closed system or other primary containment that has held viable organisms containment rDNA molecules shall not be opened for maintenance or other purposes unless it has been sterilized by a validated sterilization procedure.

vi. Emergency plants required to cover accidental spills and personnel contamination shall include methods and procedures for handling large losses of culture on an emergency basis.

4.3.1.2. BL-2LS Level

i. Cultures of viable organisms containing rDNA molecules shall be handled in a closed system (e.g., closed vessel used for propagating and growing cultures) or other primary containment equipment (e.g., Class III biological safety cabinet containing a centrifuge used to process culture fluids) designed to prevent the escape of viable organisms. Volumes less than ten (10) liters may be handled outside of a closed system or other primary containment equipment, provided all physical containment requirements specified in section 1.4 are met.

ii. Culture fluids (except as allowed in section 3.1.1.3) shall not be removed from a closed system or other primary containment equipment unless the viable organisms containing rDNA molecules have been inactivated by a validated inactivation procedure.

iii. Sample collection from a closed system, the addition of materials to a closed system and the transfer of culture fluids from one closed system to another shall be done in a manner that prevents the release of aerosols or contamination of exposed surfaces.

iv. Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters that have efficiencies close to HEPA filters or by other equivalent procedures (e.g., incineration) to prevent the release of viable organisms containing rDNA molecules to the environment.

v. A close system or other primary containment equipment that has held viable organisms containing rDNA molecules shall not be opened for maintenance or other purposes unless it has been sterilized by a validated sterilization procedure.

vi. Rotating seals and other mechanical devices directly associated with a closed system used for propagating and growing viable organisms containing rDNA molecules shall be fully enclosed in ventilated housings that are exhausted through filters that have efficiencies equivalent to HEPA filters or through other equivalent treatment devices.

vii. A closed system/containment equipment used for propagating and growing viable organisms containing rDNA molecules shall include monitoring or sensing devices that monitor the integrity of containment during operations.

viii. A closed system used for propagating and growing viable organisms containing the rDNA molecules shall be tested for integrity of the containment features using the organism that will serve as the host for propagating rDNA molecules. Testing shall be conducted before viable organisms containing rDNA molecules are introduced and after essential containment features have been modified or replaced. Procedures and methods used in the testing shall be appropriate for the equipment design and for recovery and demonstration of the test organism. Records of tests and results shall be maintained on file.

ix. A closed system used for propagating and growing viable organisms containing rDNA molecules shall be permanently identified. This identification shall be used in all record reflecting testing, operation and maintenance and in all documentation relating to use of this equipment for research or production activities involving viable organisms containing rDNA molecules.

x. The universal biohazard sign (Annex 5) shall be posted on each closed system and primary containment equipment when used to contain viable organisms containing rDNA molecules.

xi. Emergency plans required to cover accidental spills and personnel contamination shall include methods and procedures for handling large losses of culture on an emergency basis.

4.3.1.3 BL3-LS Level

i. Cultures of viable organisms containing rDNA molecules shall be handled in a closed system (e.g., closed vessels used for propagating and growing cultures) or other primary containment equipment (e.g., Class III biological safety cabinet containing centrifuge used to process culture fluids) which is designed to prevent the escape of viable organisms. Volumes less than 10 liters may be handled outside of a closed system provided all physical containment requirements specified in 5.3.1 are met.

ii. Culture fluids (except as allowed in section 3.1.1.3) shall not be removed from a closed system or other primary containment equipment unless the viable organisms containing rDNA molecules have been inactivated by a validated inactivation procedure. A validated inactivation procedure is one which has been demonstrated to be effective using the organisms that will serve as the host for propagating the rDNA molecules.

iii. Sample collection from a closed system, the addition of materials to a closed system and the transfer of culture fluids from one closed system to another shall be done in a manner which prevents the release of aerosols or contamination of exposed surfaces.

iv. Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters which have efficiencies equivalent to HEPA filters or by other equivalent procedures (e.g., incineration) to prevent the release of viable organisms containing rDNA molecules to the environment.

v. A closed system or other primary containment equipment that has held viable organisms containing rDNA molecules shall not be opened for maintenance or other purposes unless it has been sterilized by a validated sterilization procedure.

vi. A closed system used for propagating and growing viable organisms containing rDNA molecules shall be operated so that the space above the culture level will be maintained at a pressure as low as possible, consistent with equipment design, to maintain the integrity of containment features.

vii. Rotating seals and other mechanical devices directly associated with a closed system used to contain viable organisms containing rDNA molecules shall be designed to prevent leakage or shall be fully enclosed in ventilated housings that are exhausted through filters that have efficiencies equivalent to HEPA filters or through other equivalent treatment devices.

viii. A closed system used for propagating and growing viable organisms containing rDNA molecules and other primary containment equipment used to contain operations involving viable organisms containing rDNA molecules shall include monitoring or sensing devices that monitor the integrity of containment during operations.

ix. A closed system used for propagating and growing viable organisms containing rDNA molecules shall be tested for integrity of the containment features using the organisms that will serve as the host for propagating the rDNA molecules. Testing shall be conducted before viable organisms containing rDNA molecules are introduced and after essential containment features have been modified or replaced. Procedures and methods used in the testing shall be appropriate for the equipment design and for recovery and demonstration of the test organism Records of tests and results shall be maintained on file.

x. A closed system used for propagating and growing of viable organisms containing rDNA molecules shall be permanently identified. This identification shall be used in all record reflecting testing, operation and maintenance and in all documentation relating to the use of this equipment for research production activities involving viable organisms containing rDNA molecules.

xi. The Universal biohazard sign shall be posted on each closed system and primary containment equipment when used to hold viable organisms containing rDNA molecules.

xii. Emergency plans required to cover accidental spills and personnel contamination shall include methods and procedures for handling large losses of culture on an emergency basis.

xiii. Closed systems and other primary containment equipment used in handling cultures of viable organisms containing rDNA molecules shall be located within a controlled area that meets the following requirements.

a. The controlled area shall have a separate entry area. The entry area shall be a double-door space such as an air lock, anteroom or change room that separates the controlled area from the balance of the facility.

b. The surfaces of walls, ceilings and floors in the controlled area shall be such that they can be readily cleaned and decontaminated.

c. Penetrations into the controlled area shall be sealed to permit liquid or vapor phase space decontamination.

d. All utilities and service or process piping and wiring entering the controlled area shall be protected against contamination.

e. Hand-washing facilities equipped with valves that can be operated by foot, by elbow or automatically shall be located at each major work area and near each primary exit.

f. A shower facility shall be provided. This facility shall be located near the controlled area.

g. The controlled area shall be designed to preclude release of culture fluids outside the controlled area when and accidental spill or release from the closed systems or other primary containment equipment occurs.

h. The controlled area shall have a ventilation system that is capable of controlling air movement. The movement of air shall be from areas of lower contamination potential to areas of higher contamination potential. If the ventilation system provides positive pressure air supply, the system shall operate in a manner that prevents the reversal of the direction of air movement or shall be equipped with an alarm that would be activated when reversal in the direction of air movement occurs. The exhaust air from the controlled area shall not be re-circulated to other areas of the facility. The exhaust air from the controlled area may be discharged to the outdoors without filtration or other means of effectively reducing an accidental aerosol burden, provided that it can be dispersed clear of occupied buildings and air intakes.

xiv. The following personnel and operation practices shall be required:

a. Personnel entry into the controlled area shall be through the entry area specified in Section 4.3.1.3.xiii.a.

b. Persons entering the controlled area shall exchange or cover their personal clothing with work garments such as jumpsuits, laboratory coats, pants and shirts, head cover and shoes or shoe covers. On exit from the controlled area, the work clothing may be stored in a locker separate from that used for personal clothing or discarded for laundering. Clothing shall be decontaminated before laundering.

c. Entry into the controlled area when work is in progress shall be restricted to those persons required to meet program or support needs. Prior to entry, all persons shall be informed of the operating practices, emergency procedures, and the nature of the work conducted.

d. Access doors to the controlled area shall be kept closed, except as necessary for access, while work is in progress. Service doors leading directly to outdoors shall be sealed and locked while work is in progress.

e. Persons under 18 years of age shall not be permitted to enter the controlled area.

f. The universal biohazard sign shall be posted on entry doors to the controlled area and all internal doors when any work involving the organism is in progress. This includes periods when decontamination procedures are in progress. The sign posted on the entry doors to the controlled area shall include a statement of agents in use and personnel authorized to enter the controlled area.

g. Persons shall wash their hands when leaving the controlled area.

h. The controlled area shall be kept neat and clean.

i. Eating drinking, smoking and storage of food are prohibited in the controlled area.

j. An effective insect and rodent control program shall be maintained.

k. Person working in the controlled area shall be trained in emergency procedures.

l. Equipment and materials required for the management of accidents involving viable organisms containing rDNA molecules shall be available in the controlled area.

m. The controlled area shall be decontaminated in accordance with established procedures following spills or other accidental release of viable organisms containing rDNA molecules.

4.4. Biological safety cabinets

Biological safety cabinets are classified as Class I, Class II or Class III cabinets.

i. A Class I cabinet is a ventilated cabinet for personnel protection, air in it flows inward, away from the operator. The exhaust air from this cabinet filters through a HEPA filter. This cabinet is used in three operational models: (1) with full-width open front, (2) with an installed front closures panel (having four 5 inch-diameter openings) without gloves and (3) with an installed front closure panel equipped with arm-length rubber gloves. The face velocity of the inward flow of air through the full-width open front 75 feet per minute or greater.

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

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

4.5. Container requirements

i. **Plants and plant parts:**

All plants or plant parts, except seeds, cells and sub-cellular elements shall be packed in a sealed plastic bag of at least 5 mm thickness, inside a sturdy, sealed, leak proof, outer shipping container made of corrugated fiberboard, corrugated cardboard or other material of equivalent strength.

ii. **Seeds:**

All seeds shall be transported in a sealed plastic bag of at least 5 mm thickness, inside a sealed metal container. The metal container shall be capable of protecting the seeds and preventing spillage or escape. The metal container shall then be enclosed in a sturdy outer shipping container made of corrugated fiberboard, corrugated cardboard, wood or other material of equivalent strength.

iii. **Live microorganisms and/or etiologic agents, cells, or sub-cellular elements**.

All regulated materials which are live (non-inactivated) microorganisms or ethologic agents, cells or sub-cellular elements shall be packed as specified below:

a. **Volume not exceeding 50 ml.**

Regulated materials not exceeding 50 ml shall be placed in a securely closed-watertight container; primary container (test tube, vial, etc.) which shall be enclosed in a second, durable watertight container secondary container is not absolutely required case of organisms at BL1, if the total volume of all the primary containers so enclosed does not exceed 50 ml. The space at the top, bottom and sides between the primary and secondary containers shall contain sufficient non particulate absorbent material (e.g., paper towel) to absorb the entire contents of the primary containers(s) in case of breakage or leakage. Each set of primary and secondary containers shall then be enclosed in an outer shipping container made of corrugated fiberboard, corrugated cardboard, wood or other material of equivalent strength.

b. **Volume Exceeding 50 ml**

Regulated that exceed a volume of 50 ml shall comply with requirements enumerated in Section 5.3.1. In addition, a shock absorbing material, in volume at least equal to that of the absorbent material between the primary and secondary containers, shall be placed at the top, bottom and sides between the secondary container and the outer shipping container. Single primary containers shall not contain more that 1,000 ml of material. However two of more primary containers whose combined volumes do not exceed 1,000 ml may be placed in a single, secondary container. The maximum amount of microorganisms of etiologic agents, cells or sub cellular elements which may be enclosed within a single outer shipping container shall not exceed 4,000 ml.

iv. **Insects, mites and related organisms**

Insects (any life stage) shall be placed in an escape proof primary shipping container (insulated vacuum container, glass, metal, plastic, etc) and sealed to prevent escape. Such primary container shall be placed securely within a secondary shipping container of crush proof Styrofoam or other material of equivalent strength. One or more rigid ice packs may also be placed within the secondary shipping container and sufficient packing material shall be added around the primary container to prevent movement of the primary shipping container. The secondary (Styrofoam or other) container shall be placed securely within an outer shipping container made of corrugated fiberboard, corrugated cardboard, wood or other material of equivalent strength.

v. **Other macroscopic organisms**

All macroscopic organisms that are not plants and which requires continuous access to atmospheric oxygen shall be placed in primary shipping containers made of a sturdy, crush proof frame of wood, metal or material of equivalent strength, surrounded by escape proof mesh or netting of a strength and mesh size sufficient to prevent the escape of the smallest organisms in the shipment, with edges and seams of the mesh or netting sealed to prevent escape of organisms. Each primary shipping container shall be securely placed within a larger secondary shipping container made of wood, metal or equivalent strength material. The primary and secondary shipping containers shall then be placed securely within an outer shipping container made of corrugated cardboard, wood or other material of equivalent strength. The outer container may have air holes or spaces in the sides and/or ends of the container, provided that the outer shipping container must retain sufficient strength to prevent crushing of the primary and secondary shipping containers.

ANNEX-1

Guidelines for Classification of Microorganisms according to their Risk Potential:

The classification system for microorganisms has been established mainly to distinguish clearly between the small proportion of organisms potentially pathogenic to humans or other higher life forms and the huge majority of harmless organisms.

The first prerequisite for the classification is the proper identification of an organism to be used either in research or much more importantly, in a large scale biotechnological process.

The determination of the genus and species of an organism allows an initial assessment of its probable behavior as a pathogen, based on existing knowledge of the organism itself and of known species closely related to it.

In order to be able to handle a new organism properly, the assessment procedure must result in conclusive information on the safety of the organism to be used or must categorize it according to its potential risk.

Experience has shown that microorganisms isolated from the environment, e.g., from soil samples can be assumed to belong mainly to risk group I (see Table 1). Thus the process of isolation and identification of particular isolates can be performed under minimal containment conditions but applying basic hygienic measures. For identification purposes clinical material is usually handled as if it consisted of group II organisms. In any case, isolates which are processed further must be identified as soon as possible to define the appropriate safety measures for handling them.

1. Classification of wild-type strains

The classification system used today is based on a proposal of the World Health Organization in which naturally occurring microorganisms have been classified into four risk groups as summarized in Table 1 (WHO, 1983).

According to the basic consideration that risk is defined as the product of hazard and exposure, the classification of pathogenic microorganisms to risk groups II, III or, IV uses criteria such as

* the history and the known infectivity of the organism,
* the incidence of infection in the community and the presence of vectors and reservoirs,
* the virulence of the pathogen, infection dose, route of attack,
* the feasibility of immunization and the effectiveness of therapy.

The term “Pathogenicity” cannot be recorded quantitatively since its manifestation depends on many factors that vary broadly. Such factors are, for example, the actual genetic constitution or physiological state of both the infections agent and the target organism. Furthermore, the resistance or susceptibility of a microorganism to adverse conditions such as temperature, pH, moisture and the like influence the chances of possible infections.

Table 1. Classification of infective Microorganisms by risk groups requirement of Biosafety levels (WHO), Laboratory Biosafety Manual, 1983:

Risk Groups	Pathogenicity features
Risk Group I (low individual and community risk) (BL-1)	A microorganism that is unlikely to cause human disease or animal disease of veterinary importance.
Risk Group II (moderate individual risk, limited community risk) (BL-2)	A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock, or the environment Laboratory exposure may cause serious infection but effective treatment and preventive measures are available and the risk of spread is limited.
Risk Group III (high individual, low community risk) BL-3)	A pathogen that usually produces serious human disease but does not ordinarily spread from one infected individual ~~of~~ to another.
Risk Group IV (high individual and community risk) (BL-4)	A pathogen that usually produces serious human or animal disease and may be readily transmitted from one individual to another, directly or indirectly.

Although there is a worldwide agreement that the four-class classification system is sufficient to meet the intended objectives, there have been differences in the description of risk classes in various national lists. In an attempt to harmonize the situation, the Working Party on Safety in Biotechnology of the European Federation of Biotechnology (EFB) proposed a "consensus" description for the classification of microorganisms according to their pathogenicity (Table-2). Table-2 contains also another proposal of the above mentioned Working Party, namely to use descriptive adjectives instead of or in addition to numbers to avoid possible confusions with different numbering systems. The special group E mentioned in Table-2 is discussed below.

Table- 2. EFB Classification of microorganisms according to pathogenicity :

Class Name	Pathogenicity features
Class I : Harmless	This class contains those microorganisms that have never been identified as causative agents of disease in man and that offer no threat to the environment. They are not listed in higher classes or in Group E.
Class II : Low Risk	This class contains those microorganisms that may cause disease in man and which might therefore offer hazard to laboratory workers. They are unlikely to spread in the environment. Prophylactics are available and treatment is effective.
Class III : Medium Risk	This class contains those microorganisms that offer severe threat of the health of laboratory workers but comparatively small risk to the population at large. Prophylactics are available and treatment is effective.
Class IV: High Risk	This class contains those microorganisms that cause severe illness in man and offer a serious hazard to laboratory workers and people at large. In general effective prophylactics are not available and no effective treatment is known.
Class E : Environmental Risk	This group contains microorganisms that offer a more severe threat to the environment than to man. They may be responsible for heavy economic losses. National and international lists and regulations concerning these microorganisms are already in existence in contexts other than biotechnology (e.g., for phytosanitary purposes).

It should be clearly noted that class I is described as “harmless”, which does not mean “no risk at all”. Some of these microorganisms may induce diseases under special circumstances, e.g., in immuno-deficient individuals. For obvious reasons, in large-scale processes microorganisms of intrinsic low risk, i.e., the harmless ones, should be used whenever possible. In fact, a number of class I organisms have a history of extended safe use in biotechnological industry as well as in human consumption or in agricultural applications.

Class II organisms are all classified as pathogens in the context of hospital, clinical and diagnostic laboratories. The scale of operation is rather small compared to most other industrial biotechnological processes.

Some of class II organisms are normally present in the environment and even in food at relatively high concentrations without offering any threat to normal persons (Collins 1990). Medium risk microorganisms (class III) are rarely used in industry, although established and tested containment systems are available to handle them safely, e.g., in vaccine production. There is no industrial use of class IV, the high-risk organisms. Worldwide only a few specialized laboratories are properly installed and equipped for their small-scale use.

For obvious reasons, there is a strong tendency to replace medium or high risk operations by harmless techniques using the possibilities offered by the methodology of genetic engineering; the well known example of vaccine production using cloned antigens instead of wild-type pathogens demonstrates this clearly.

The classification system described so far has also been applied without problems to wild-type microorganisms that have been modified using the traditional genetic techniques of mutagenesis and selection. To our knowledge, in no case had an organism to be transferred to a higher risk class than the wild type after mutagenesis. However, there is a very popular example for the contrary: Escherichia coli wild type is in risk class II as a potential human pathogen, whereas its derivative E. coli K12 is classified as harmless in all national lists.

With a few exceptions, human pathogens are relatively easy to categorize in such lists. The situation is much more complex with plant or animal pathogens, the potential risks of which may greatly depend on local situations.

As possible step towards a solution of the problems of classification of microorganisms that are harmless to man but may be pathogenic for other higher life forms, in Table 2 a special group E was established that contains microorganisms of environmental risk. As far as plant pathogens are concerned, this group has been defined further (Table 3). Adequate containment conditions have also been elaborated describing three safety levels, one for each of the classes shown in Table-3, aimed at minimizing any adverse effects that might result from the use of plant pathogens in laboratories and in large-scale processing facilities.

Table-3. EFB Classification of microorganisms pathogenic for plants :

Class Names	Pathogenicity features
Class Ep. 1	This class contains those microorganisms which may cause disease in plants but have local significance only. They may be mentioned in a list of pathogens for individual countries concerned. Very often they are endemic pathogens for plants and do not require any special physical containment. However, it may be advisable to employ good microbiological techniques (GMT).
Class Ep. 2	Microorganisms known to cause outbreaks of disease in crops as well as in ornamental plants. These pathogens are subject to regulations for species listed by authorities of the country concerned
Class Ep. 3	Microorganisms mentioned in quarantine lists. Importation and handling of these organisms are generally forbidden. The regulatory authorities must be consulted by prospective users.

From Frommer and Kramer (1990)

1.1 **Good Microbiological Techniques (GMT):** A summary of the GMT is presented in Box 1.

Box -1: Good Microbiological Techniques (GMT) for the safe handling of microorganisms with risk potential:

These techniques are intended to protect both the operator and product.

1. The operator should have basic knowledge of microbiology. Spreading of pathogens should not occur, e.g., via contaminated surfaces, hands or clothes. All workers should be aware of the risks of cultivated pathogens to people in the vicinity. Entry to the working place should be confined to persons who are aware of these risks.
2. There should be no contact between materials or tools in the working place and the mouth of the operator. Eating, drinking and mouth pipetting are not allowed.
3. No activities which may produce aerosols are permitted at the working place: centrifuging, blending, filling of bottles or tubes should be carried out in a Biosafety cabinet.
4. Infected waste should be placed in sealable containers, the outside of which should be disinfected before transport to the autoclave or incinerator.
5. Heat or chemical sterilization processes should be investigated beforehand to ensure that the required killing rate in obtained.
6. Reliable equipment should be used.
7. Working surfaces, tables and hands should be disinfected after normal working.
8. Working surfaces, tables, floors and hands should be disinfected after spillage of infectious material.
9. In case of accidents, an emergency scheme with details of first aid, cleaning and disinfection should be available, and the staff should be trained accordingly.

2. Classification of recombinant-DNA organisms

There has been a long and sometimes controversial debate about the risk potentials and the classification of organisms modified by recombinant DNA techniques. The discussions resulted in many countries in the elaboration and implementation of laws or other legal regulations specifically dealing with genetic engineering. Nevertheless, it is now generally accepted that genetically modified or engineered organisms must not at all be regarded as hazardous per se, but can be classified into the same four-class system used for wild-type organisms. Provided there is sufficient knowledge of the recipient, the origin and nature of the introduced gene(s) and the vector used, a reliable risk assessment can be carried out for the organisms modified by recombinant-DNA techniques.

This is one of the major conclusions that can be drawn from almost 20 years of experience with the recombinant-DNA methodology in which no basically unexpected or quantitative novel risks have been realized.

The basic statement of the WHO Biosafety Manual is still valid, and there is no reason to assume that this will change: “There are no unique or specific risks associated with recombinant DNA work (genetic engineering), the risks are not greater than those associated with work with known pathogens and do not necessitate special laboratory design or practice” (WHO 1983). That means that all sorts of guidelines and regulations set for work with microorganisms and other biological agents can be transferred accordingly to work with recombinant DNA organisms.

This general statement has been broadly accepted worldwide, also in those countries in which genetic engineering by specific laws.

3. Good occupational safety and hygiene

The term Good Occupational Safety and Hygiene summarizes the normal hygiene procedures applied in large-scale biotechnological processes with microorganisms of group I in which both the contamination of the process itself by microorganisms or other substances must be avoided, as well as their transfer to the personnel and the workplace; or where such transfer can be minimized.

Article 7 of the EC Council Directive on the contained use of genetically modified organisms (90/219/EEC), which reproduces the OECD recommendations of 1986 literally, specifies the following principles for working with group 1 organisms (In the new version of the OECD Report "Safety Considerations for the use of Genetically Modified Organisms" of 1992, some points are further elaborated, and the concept has been renamed "Fundamental Principles of Good Occupational and Environmental Safety" major supplements are given in parentheses**)**

1. to keep workplace and environmental exposure to any physical, chemical, or biological agent (including cellular products and debris) at the lowest practicable level (to a level appropriate to the characteristics of the organisms, the product and the process);
2. to exercise engineering control measures at source and to supplement these with appropriate personal protective clothing and equipment when necessary;
3. to test adequately and maintain control measures and equipment. (The frequency of examination and testing will depend on the nature of the modified organisms, the product and the process);
4. to test, when necessary, for the presence of viable process organisms outside the primary physical containment (outside the process equipment, both in the workplace and in the environment);
5. to provide training of personnel;
6. to establish biological safety committees or subcommittees as required (and/or to consult with worker representatives and to consult with regulatory authorities);
7. to formulate and implement local codes of practice for the safety of personnel (and for the protection of the environment).

ANNEX-2

Good Laboratory Practice

For any laboratory dealing with biotechnological research a routine maintenance program ensures conditions safe for both workers and scientists of the laboratory and the environment. To ensure this, universally accepted laboratory practices are in use in the laboratories dealing with biotechnological research in different parts of the world. So the criteria of good laboratory practice have been given in detail in this chapter.

1. Test Facility Organization and Personnel

1.1. Management's Responsibilities

A. Test facility management should ensure that the Principles of Good Laboratory Practice are complied within the test facility
B. At a minimum it should
   i. ensure that qualified personnel, appropriate facilities, equipment and materials are available.
   ii. maintain a record of the qualifications, training, experience and job description for each professional and technical individual;
   iii. ensure that personnel clearly understand the functions they are to perform and, where necessary, provide training for these functions;
   iv. ensure that health and safety precautions are applied according to national and/or international regulations;
   v. ensure that appropriate Standard Operating Procedures are established and followed;
   vi. ensure that there is a Quality Assurance Programme with designated personnel;
   vii. where appropriate, agree to the study plan in conjunction with the sponsor;
   viii. ensure that amendments to the study plan are agreed upon and documented;
   ix. maintain copies of all study plans;
   x. maintain a historical file of all Standard Operating Procedures;
   xi. for each study ensure that a sufficient number of personnel is available for its timely and proper conduct;
   xii. for each study designate an individual with the appropriate qualifications, training, and experience as the Study Director before the study is initiated. If it is necessary to replace a Study Director during a study, this should be documented;
   xiii. ensure that an individual is identified as responsible for the management of the archives;

1.2. Study Director's responsibilities

A. The Study Director has the responsibility for the overall conduct of the study and for its report.
B. These responsibilities should include, but not be limited to, the following functions:
   i. should agree to the study plan;
   ii. ensure that the procedures specified in the study plan are followed and that authorization for any modification is obtained and documented together with the reasons for them;
   iii. ensure that all data generated are fully documented and recorded;
   iv. sign and date the final report to indicate acceptance of responsibility for the validity of the data and to confirm compliance with these Principles of Good Laboratory Practice.
   v. ensure that after termination of the study, the study plan, the final report, raw data and supporting material are transferred to the archives.

1.3. **Personnel responsibilities**

A. Personnel should exercise safe working practice. Chemicals should be handled with suitable caution until their hazard(s) has been established. Special precaution should be exercised by pregnant women.
B. Personnel should exercise health precautions to minimize risk to themselves and to ensure the integrity of the study.
C. Personnel known to have a health or medical condition that is likely to have an adverse effect on the study should be excluded from operations that may affect the study.

2. **Quality assurance program**

2.1. **General**

A. The test facility should have a documented quality assurance program to ensure that studies performed are in compliance with these Principles of Good Laboratory Practice.

B. The quality assurance program should be carried out by an individual or by individuals designated by and directly responsible to management and who are familiar with the test procedures.

C. This individual(s) should not be involved in the conduct of study being assured.

D. This individual(s) should report any findings in writing directly to management and to the Study Director.

2.2. **Responsibilities of the quality assurance personnel**

The responsibilities of the quality assurance personnel should include, but not be limited to, the following functions:

i. ascertain that the study plan and Standard Operating Procedures are available to personnel conducting the study;
ii. ensure that the study plan and Standard Operating Procedures are followed by periodic inspections of the test facility and/or by auditing the study in progress. Records of such procedures should be retained;
iii. promptly report to management and the Study Director unauthorized deviations from the study plan and from Standard Operating Procedures;
iv. review the final reports to confirm that the methods, procedures, and observations are accurately described and that the reported results accurately reflect the raw data of the study;
v. prepare and sign a statement, to be included with that final report, which specifies the dates inspections were made and the dates any findings were reported to management and to the Study Director.

3. **Facilities**

3.1. **General**

A. The test facility should be of suitable size, construction and location to meet the requirements of the study and minimize disturbances that would interfere with the validity of the study.

B. The design of the test facility should provide an adequate degree of separation of the different activities to assure the proper conduct of each study.

3.4 **Archive facilities**

Space should be provided for archives for the storage and retrieval of raw data, reports, samples and specimens.

3.5 **Waste disposal**

A. Handling and disposal of wastes should be carried out in such a way as not to jeopardize the integrity of studies in progress.

B. The handling and disposal of wastes generated during the performance of a study should be carried out in a manner which is consistent with pertinent regulatory requirements. This would include provision for appropriate collection, storage and disposal facilities, decontamination and transportation procedures and the maintenance of records related to the preceding activities.

4. **Apparatus, material and reagents**

4.1. **Apparatus**

A. Apparatus used for the generation of data and for controlling environmental factors relevant to the study should be suitably located and an appropriate design and adequate capacity.

B. Apparatus used in a study should be periodically inspected, cleaned, maintained, and calibrated according to Standard Operating Procedures. Records of procedures should be maintained.

4.2. **Material**

Apparatus and materials used in studies should not interfere with the test systems.

4.3. **Reagents**

Reagents should be labeled, as appropriate, to indicate source, identity, concentration and stability information and should include the preparation date, earliest expiration date, specific storage instructions.

5. **Test systems**

5.1. **Physical/Chemical**

A. Apparatus used for the generation of physical/chemical data should be suitably located and of appropriate design and adequate capacity.

B. Reference substances should be used to assist in ensuring the integrity of the physical/chemical test systems.

5.2. **Biological**

A. Proper conditions should be established and maintained for the housing, handling and care of animals, plants, microbial as well as other cellular and sub-cellular systems, in order to ensure the quality of the data.

B. In addition, conditions should comply with appropriate national regulatory requirements for the import, collection, care and use of animals, plants, microbial as well as other cellular and sub-cellular systems.

C. Newly received animal and plant test systems should be isolated until their health status has been evaluated. If any unusual mortality or morbidity occurs, this lot should not be used in studies and when appropriate, humanely destroyed.

D. Records of source, date of arrival and arrival condition should be maintained.

E. Animal, plant, microbial and cellular test systems should be acclimatized to the test environment for an adequate period before a study is initiated.

F. All information needed to properly identify the test systems should appear on their housing or containers.

G. The diagnosis and treatment of any disease before or during a study should be recorded.

6. **Test and reference substances**

6.1 **Receipt, handling, sampling and storage**

A. Records including substance characterization, date of receipt, quantities received and used in studies should be maintained.

B. Handling, sampling and storage procedures should be identified in order that the homogeneity and stability is assured to the degree possible and contamination or mix-up are precluded.

C. Storage container(s) should carry identification information, earliest expiration date and specific storage instructions.

6.2. **Characterization**

A. Each test and reference substance should be appropriately identified (e.g. code, chemical abstract number (CAS), name).

B. For each study, the identity, including batch number, purity, composition, concentrations, or other characterizations to appropriately define each batch of the test or reference substances should be known.

C. The stability of test and reference substances under conditions of storage should be known for all studies.

D. The stability of test and reference substances under the test conditions should be known for all studies.

E. If the test substance is administered in a vehicle, Standard Operating Procedures should be established for testing the homogeneity and stability of the test substance in that vehicle.

F. A sample for analytical purposes for each batch of test substance should be regained for studies in which the test substance is tested longer than four weeks.

7. Standard operating procedures

7.1. **General**

A. A test facility should have written Standard Operating Procedures approved by management that are intended to ensure the quality and integrity of the data generated in the course of the study.

B. Each separate laboratory unit should be immediately available. Standard Operating Procedures relevant to the activities being performed therein. Published text books, articles and manuals may be used as supplements to these Standard Operating Procedures.

7.2. **Application**

Standard Operating Procedures should be available for, but not be limited to, the following categories of laboratory activities. The details given under each heading are to be considered as illustrative examples.

A. Test and Reference Substance:
   Receipt, identification, labeling, handling, sampling and storage.
B. Use, maintenance, cleaning, calibration of measuring apparatus and environmental control equipment, preparation of reagents.
C. Record keeping, reporting, storage and retrieval:
   Coding of studies, data collection, preparation for reports, indexing systems, handling of data including the use of computerized data systems.
D. Test system (where appropriate)
   i. Room preparation and environmental room conditions for the test system.
   ii. Procedures for receipt, transfer, proper placement, characterization, identification and care of test system.
   iii. Test system preparation, observations examinations, before, during and at termination of the study.
   iv. Handling of test system individuals found moribund or dead during the study.
   v. Collection, identification and handling of specimens including necropsy and histopathology.
E. Quality Assurance procedures: Operation of quality assurance personnel in performing and reporting study audits, inspections and final study report reviews.
F. Health and Safety Precautions: as required by national and/ or international legislation or guidelines.

8. **Performance of the study**

8.1. **Study plan**

A. For each study, a plan should exist in a written form prior to initiation of the study.
B. The study plan should be retained as raw data.
C. All changes, modifications or revisions of the study plan, as agreed to by the Study Director, including justification(s), should be documented, signed and dated by the Study Director, and maintained with the study plan.

8.2. **Content of the study plan**

The study plan should contain, but not be limited to the following information:

A. Identification of the study, the Test and Reference Substance;
   i. A descriptive title;
   ii. A statement which reveals the nature and purpose of the study;
   iii. Identification of the test substance by code or name (IUPAC, CAS number etc);
   iv. The reference substance to be used.

B. Information Concerning the Sponsor and the Test Facility
   i. Name and address of the Sponsor;
   ii. Name and Address of the Test Facility;
   iii. Name and address of the Study Director.

C. Dates
   i. The date of agreement to the study plan by signature of the study Director, and when appropriate, of the sponsor and/or the test facility management;
   ii. The proposed starting and completion dates.

D. Test Methods
   i. Reference to OECD Test Guideline or other test guideline to be used.

E. Issues (where applicable)
   i. The justification for selection of the test system;

ii. Characterization of the test system, such as the species, strain, sub-strain, source of supply, number, body weight range, sex, age, and other pertinent information.
iii. The method of administration and the reason for its choice;
iv. The dose levels and/or concentration(s), frequency, duration of administration;
v. Detailed information on the experimental design, including a description of the chronological procedure of the study, all methods, materials and conditions, type and frequency of analysis, measurements, observations and examinations to be performed.

F. Records: a list of records to be retained.

8.3. **Conduct of the study**

A A unique identification should be given to each study. All items concerning this study should carry this identification.
B. The study should be conducted in accordance with the study plan.
C. All data generated during the conduct of the study should be recorded directly, promptly, accurately and legibly by the individual entering the data. These entries should be signed or initialed and dated.
D. Any change in the raw data should be made so as not to obscure the previous entry, and should indicate the reason, if necessary, for change and should be identified by date and signed by the individual making the change.
E. Data generated as a direct computer input should be identified at the time of data input by the individual(s) responsible for direct data entries. Corrections should be entered separately by the reason for change, with the date and the identity of the individual making the change.

9. **Reporting of study results**

9.1. **General**

A. A final report should be prepared for the study.
B. The use of the International System of Units (SL) is recommended.
C. The final report should be signed and dated by the Study Director.
D. If reports of principal scientists from co-operating disciplines are included in the final report, they should sing and date them.
E. Corrections and additions to a final report should be in the form of an amendment. The amendment should clearly specify the season for the corrections or additions and should be signed and dated by the Study Director and by the principal scientist from each discipline involved.

9.2. **Content of the final report**

The final report should include but not be limited to the following information :

A. Identification of the Study, the Test and Reference Substance
   i. A descriptive title.
   ii. Identification of the test substance by code or name (IUPAC CAS number, etc.);
   iii. Identification of the reference substance by chemical name;
   iv. Characterization of the test substance including purity, stability and homogeneity.

B. Information Concerning the Test Facility
   i. Name and address;
   ii. Name of the Study Director
   iii. Name of the principal personnel having contributed reports to the final report.
C. Duties
   i. Dates on which the study was initiated and completed.

D. Statement
   i. A Quality Assurance statement certifying the dates inspections were made and the dates any findings were reported to management and to the Study Director.
E. Description of Materials and Test Methods
   i. Description of methods and materials used.
   ii. Reference to OECD Test Guidelines or other test guidelines.
F. Results
   i. A summary of results;
   ii. All information and data required in the study plan;
   iii. A presentation of the results, including calculations and statistical methods;
   iv. An evaluation and discussion of the results and, where appropriate, conclusions.
G. Storage
   i. The location where all samples, specimens, raw data and the final report are to be stored.

10.**Storage and retention of records and material**

10.1. **Storage and retrieval**

A. Archives should be designed and equipped for the accommodation and the secure storage of the following :
   i. The study plans.
   ii. The raw data.
   iii. The final reports.
   iv. The reports of laboratory inspections and study audits performed according to the Quality Assurance Program.
   v. Sample and specimens.
B. Materials retained in the archives should be indexed so as to facilitate orderly storage and rapid retrieval.
C. Only personnel authorized by management should have access to the archives. Movement of material in and out of the archives should be properly recorded.

10.2. **Retention**

A. The following should be retained for the period specified by the appropriate authorities:
   i. The study plan, raw data, samples, specimens, and the final report of each study;
   ii. Records of all inspections and audits performed by the Quality Assurance Program;
   iii. Summary of qualifications, training, experience and job descriptions of personnel;
   iv. Records and reports of the maintenance and calibration of equipment;
   v. The historical file of Standard Operation Procedures.
B. Samples and specimens should be retained only as long as the quality of the preparation permits evaluation.
C. If a test facility or an archive contracting facility goes out of business and has no legal successor, the archive should be transferred to the archives of the sponsor(s) of the study(s).

ANNEX- 3

Good Industrial Large-Scale Practice: The GILSP Concept

According to the Report "Recombinant DNA Safety Considerations" (OECD, 1986) a catalog of certain criteria has been suggested to classify a genetically modified organisms into the lowest risk group (Tables 5 and 6). According to these recommendations, a recombinant-DNA organism of intrinsically low risk can be handled under conditions of "good industrial large scale practice" (GILSP), i.e., under the same conditions of minimal control and containment procedures as they would be used for the harmless host strain from which it is derived.

Table-4: Criteria for classifying genetically modified microorganisms in Group I

Sl.	Types of Organism	Criteria
A	**Recipient or Parenteral Organism**	i. Non-pathogenic ii. No adventitious agents iii. Proven and extended history of safe use or built-in biological barriers, which, without interfering with optimal growth in the reactor or fermentor, confer limited survivability and replicability, without adverse consequences in the environments.
B	**Vector/Insert**	i. Well characterized and free from known harmful sequences ii. Limited in size as much as possible to the genetic sequences required to perform the intended function. iii. Should be poorly mobilizable iv. Should not transfer any resistance markers to microorganisms not known to acquire them naturally (if such acquisition could compromise use of drug to control disease agents)
C	**Genetically Modified Microorganisms**	i. Non-pathogenic ii. As safe in the reactor or fermentor as recipient of parenteral organisms, but with limited survivability and/or replicability without adverse consequences in the environment.
D	**Other Genetically Modified microorganisms** that could be included in Group I, if they meet the conditions in C above.	i. Those constructed entirely from a single prokaryotic recipient (including its indigenous plasmids and viruses) or form a single eukaryotic recipient (including its chloroplasts, mitochondria, plasmids but excluding viruses. ii. Those that consist entirely of genetic sequences from different species that exchange these sequences by known physiological processes.

Table-5. Relationship between risk class, containment category and objectives of safety precautions.

Risk classes	Containment category (OECD)	Safety Objectives
Class 1	GILSP	Safeguard hygiene for work with harmless microorganisms which do not require containment. Hygienic process and equipment are used to prevent the contamination of culture or product.
Class 2	C1	Minimize the release of low-risk microorganisms from primary containment, no secondary containment.
Class 3	C2	Prevent release of medium-risk microorganisms from primary containment during regular operations; no strict secondary containment.
Class 4	C3	Absolute cont-ainment for high-risk microorganisms; secondary containment strictly requbired to prevent release in case of breach of

		primary containment.

Table-6: Relationship between risk class, containment category and safety precautions.

Risk Classes	**Types of Organisms**	**Safety precautions**
Class 1	Harmless microorganisms	- GILSP - Good occupational safety and hygiene principles are to be applied
Class 2	Low risk microorganisms	Containment category I - GMT are to be applied - Surfaces within the facility should be easily cleaned and disinfected - Contaminated materials are to be autoclaved or disinfected before cleaning - Aerosol creating procedures must be controlled and contained - Access to the facility must be restricted
Class 3	Medium risk microorganisms	Containment category II Measure in addition to containment I conditions : - Only authorized personnel is admitted to the facility - Personnel is vaccinated if possible - Exhaust air is HEPA filtered - Effluents from the facility must be decontaminated or sterilized - An autoclave should be present within the facility - All processes involving medium-risk microorganisms must be carried out in hermetically sealed equipment or in Biosafety cabinets - Protective suits, closing at the back, have to be worn by personnel - Hands and forearms should be washed and disinfected at regular intervals
Class 4	High Risk Microorganisms	Containment category 3 Measures in addition to containment 2 conditions : - No visitors should be admitted - Facility must be completely isolated - The room for complete change of clothes must include an air-lock facility with compulsory shower - Negative pressure must be maintained in the facility and the air ducts must be protected by double HEPA filters - All effluents must be sterilized - The autoclave must be within the facility - Materials containing high-risk microorganisms must be absolutely separated from workers - Protective suits for single use must be worn

It is anticipated that the vast majority of biotechnological processes will be designed to use organisms that merit the designation GILSP. This is not only to ensure the safety of workers and of the environment but, of course, also to avoid the need for costly physical containment measures.

Table- 7: Safety precautions for biotechnological operations.

Measures, Operations, Equipment Design, Category Facilities	GILSP	Containment		
		1	2	3
Procedure				
Written instructions and code of practice	+	+	+	+
Biosafety manual	-	+	+	+
Good occupational hygiene	+	+	+	+
Gppd ,ocrpbop;pgoca; tecjmoqies	-	+	+	+
Biohazard sign	-	+	+	+
Restricted access	-	+	+	+
Accident reporting	+	+	+	+
Medical surveillance	-	+	+	+
Primary Containment : Operation and Equipment Closed system (CS) designed to minimize (m) of				
prevent (p) the release of viable microorganisms	-	m	p	p
Treatment of exhaust air or gas from CS	-	m	p	p
Sampling from CS	-	m	p	p
Addition of materials to CS	-	m	p	p
Removal of materials, products, effluents from CS	-	m	p	p
Penetration of CS by agitator shaft and measuring devices	-	m	p	p
Foam-out control	-	m	p	p
Secondary containment : Facilities				
Protective clothing according to risk category	+	+	+	+
Changing/washing facility	+	+	+	+
Disaffection facility	-	+	+	+
Emergency shower facility	-	-	+	+
Airlock and compulsory shower facility	-	-	-	+
Effluents decontaminated	-	-	+	+
Controlled negative pressure	-	-	-	+
HEPA filters in air ducts	-	-	+	+
Tank for spilled fluids	-	-	+	+
Area hermetically scalable	-	-	-	+

+ = required, - = not required; CS-closed system; m-minimize release; p = prevent release.

Classification of safety measures: t**he four-class safety precaution system**

If the relevant information about the properties of an organism is available, the procedures of handling this organism together with appropriate containment facilities, if necessary, can be categorized. The internationally accepted four-class risk classification system has been logically related to a four-part safety precaution system both for biotechnological laboratories and industrial processes (see Tables 7 and 8).
Despite the still existing differences in the numbering system, there is a broad conformity in the contents of the various nationally or internationally used lists of containment levels. In the following table (Table 9) containment procedures recommended for Industrial Processes for containment levels 2 to 4 are summarized.

Table-8: Containment measures for industrial processes with biological agents.

	Containment measures	Containment levels		
		2	3	4
1.	Viable organisms should be handled in a system which physically separates the process from the environment	Yes	Yes	Yes
2.	Exhaust gases from the closed system should treated so as to:	Minimize release	prevent release	Prevent release
3.	Sample collection, addition of materials to a closed system and transfer of viable organisms to another closed system, should be performed.	Minimize release	prevent release	Prevent release
4.	Bulk culture fluids should not be removed from the closed system unless the viable organisms have been:	Inactivated by validated means	Inactivated by validated chemical or physical means	Inactivated by validated chemical or physical means
5.	Seals should be designed so as to :	Minimize release	prevent release	Prevent release
	a) Biohazard signs should be posted	Optional	Yes	Yes
	b) Access should be restricted to nominated personnel only	Yes	Yes	Yes, via an airlock
	c) Personnel should wear protective clothing	Yes	Yes	A complete change
	d) Decontamination and washing facilities should be provided for personnel	Yes	Yes	Yes
	e) Personnel should shower before leaving the controlled area	No	Optional	Yes
	f) Effluent from sinks and showers should be collected and inactivated before release	No	Optional	Yes
	g) The controlled area should be adequately ventilated to minimize air contamination	Optional	Optional	Yes
	h) The controlled area should be maintained at an air pressure negative to atmosphere	No	Optional	Yes
	i) Input air to and exit air from the controlled area should be HEPA filtered	No	Optional	Yes
	j) The controlled area should be designed to contain spillage of the entire contents of the closed system	No	Optional	Yes
	k) The controlled area should be sealable to permit fumigation	No	Optional	Yes
	l) Effluent treatment before final discharge	Inactivated by validated means	Inactivated by validated chemical or physical means	Inactivated by validated chemical or physical means

ANNEX-4

List of Organisms According to Different Risk Groups

Safety considerations in the application of biotechnology are imperative since possible risk in research and development involving different microorganisms has been recognized. According to their possible risks to health and environment the microorganisms have been classified in to different risk groups.

BACTERIA
Risk group I

Risk group I contains organisms as per definition cause no risk for health and environment. However, GLP guidelines have to be followed. The list should ease the grouping and identification of specific strains. It does not compensate the responsibility of the scientists.
Acetobacter spp.
Actinoplanes spp.
Agrobacterium spp.
Alcaligenes aquamarinus/eutrophus/latus
Aquaspirillum spp.
Arthrobacter spp.
Azotobacter spp.
Bacillus spp., except cereus and anthracis
Bifidobacterium.spp., except dentium
Brdyrhizobium spp.
Brevibacterium spp.
Caryphanon spp.
Clavibacter spp. Except michiganensis and sepedonicus
Clostridium
aceticum/acetobutylicum/acidiurici/cellobiparum/kluyveri/thermoaceticum/thermocellum/thermosulfurogenes
Corynebacterium glutomicum/lilium
Enterococcus facium ATCC 4043
Escherichia coli ATCC 9637, CCM28, NCIB 8743, B, K12 and derivatives
Erwinia spp. Except chrysanthemi, amylovora and herbicola
Gluconobacter
Klebsiella planticola
Lactobacillus
acidophilus/bauaricus/breuis/bucneri/casei/cellobiosis/fermentum/fermentum/helveticum/sake
Lactococcus lactis
Leuconostoc spp.
Lysobacter spp.
Methanobacter spp.
Methylomonas spp.
Micrococcus spp.
Pediococcus spp.
Pseudomonas gladioli/fluorescens /syringae, except pathotype persicae
Rhizobium spp.
Rhodobacter spp.
Rhodopseudomonas spp.
Staphylococcus carnosus
Rickettsiella spp.
Streptococcus salivarius-thermophilus
Streptomyces spp., except somaliensis
Thermobacteroides spp.

Thermus spp.
Thiobacillus spp.
Vibrio diazotrophicus/fischeri

BACTERIA
Risk group II

Risk group II contains pathogenic bacteria for which respective therapy is available or which infective character is less severe or for which easy-to-use contamination procedures are available (i.e. do not form spores etc.), moderate to high safety level (2-3).
Actinobacillus pp.
Actinomyces bovis/israelii/pyrogenes
Aeromonas hydrophilla
Anaplasma spp.
Arcanobacterium haemolyticum
Bacteroides fragilis/thetaiotaomicron
Bifidobacterium dentium
Bordetella spp.
Borrelia spp.
Campylobacter spp.
Citrobacter spp.
Clamydia trachomatis
Clostridium botulinum/chauvoei/haemolyticum/histolyticum/ novyi/perfringens/septicum/tetani
Corynebacterium diphtheriae/pseudotuberculosis/renale/ulcerans
Cytophaga spp. pathogenic to animals
Edwardsiella tarda
Eikenella coreodens
Enterobacter spp.
Enterococcus faecalis
Eperythrozoon spp.
Erysipelothrix rhusiopathiae/tonsillarum
Escherichia coli (enteroinvasive, enteropathogenic, enterotoxic etc. strains)
Flavobacterium meningosepticum
Haemophillus spp.
Klebsiella, Pathogenic spp.
Legionella spp.
Letospira interrogans
Listeria monocytogenes
Moraxella spp.
Neisseria gonorrhoeae/meningitidis
Nocardia brasiliensis/asteroides/farcinica/nova
Pantoea agglomerans
Pasteurella spp.
Peptococcus spp.
Peptostreptococcus spp.
Proteus spp.
Providencia spp.
Pseudomonas aeruginosa/pseucomallei
Rhodococcus equi
Salmonella spp.
Serratia marcescens
Shigella spp.
Staphylococcus aureus

Streptobacillus moniliformis
Streptococcus pyogenes/pneumoniae
Streptomyces somaliensis
Ureaplasma urealyticum
Veillonella spp.
Vibrio cholerae/fluvialis/metschnikovii/mimicus/parahaemolyticus/vulnificus
Yersinia spp., except pestis

BACTERIA
Risk group III

Risk group III contains pathogenic bacteria with high infective potential, no or less efficient therapy or resistant forms (like spores), highest safety level (3-4).
Bacillus anthracis
Bartonella bacilliformis
Brucella melitensis
Chalamydia psittaci
Coxiella burnetii
Francisecla tularensis
Mycobacterium africanum/tuberculosis
Mycobacterium avium/chelonae/fortuitum/marinum/scrofuiaceum/ulcerans
Mycoplasma spp.
Pseudomanas mallei
Rickettsia
akari/australis/bellii/canada/conori/montana/parkeri/sibirica/tsutsugamushi/typhi
Treponema pallidum/pertenue
Yersinia pestis

FUNGI
Risk group I

Agaricus bisporus
Acremonium chrysogenum/strictum/elegans
Actinomucor elegans
Ashyba gossypii
Aspergillus oryzae
Aureobasidum pullulans
Blakeslea trispora
Brettanomyces bruxellensis
Candida boindinii/shehateae/utilis
Chaetonium globosum
Cladosporium cladosporioides
Claviceps paspali/purpurea
Coprinus cinereus
Cunninghamella blakesleana/elegans
Curvularia lunate
Cyathus stercoreus
Debaryomyces hansenii
Dacrymyces deliquescens
Engyodontium album
Geotrichum candidum
Hansenula anomala/polymorpha
Hypholama fasciculare/roseonigra

Kloecdera corticis
Lentinus edodes
Lipomyces lipofer/sarkeyi
Metarhizium anisopliae
Monascus pupureus/ruber
Moniliella suaveolena
Mortierella vinacea
Mucor circnelloides/mucedo/plumbeus/rouxii
Myrothecium verrucaria
Neurospora crassa/sitophilla
Nigrospora sphaerica
Oxyporus populinus
Pachysolen tannophilus
Paecilomyces varioti/lilacinus
Penicillium camemberti/chrysogenum/funiculosum
Phycomyces blakesleanus
Pichia farinosa/guilliermondii/membranae faciens/stipitis
Pleurotus ostreatus
rhizoctonia solani
Rhizopus oryzae/stolonifer
Rhodosporidum toruloides
Rhodotorual glutinis
Saccharomyces cerevisiae
Schizoasccharomyces pombe
Schwanniomyces occidentalis
Sordaria macrpsopra
Thanatephorus cucumeris
Trametes vesicolor
Trichoderma harzianum/longibrachiatum/viridae
Trigonopsis variabilis
Verticillium lecanii
Volvariella volvacea
Wallernia sebi
Xeromyces bisporus
Zygorhynus moelleri
Zygosaccharomyces bailii/rouxii

FUNGI
Risk group II

Acremonium falsiforme/kiliense/recifei
Arthroderma benhamiae/simiti
Aspergillus flavus/fumigatus
Basidiobolus haptosporus
Candida albicans
Cryptococcus neoformans
Epidermophyton floccosum
Exophilia castelanii/dermatitidis/mansonii
Filobasidiella neoformans
Fonsecaea compacta
Hortaea werneckii
Leptoshaeria senegalensis/thompkinsii
Loboa loboi

Madurella grisea/mycetomi
Microsporum audouinii/canis/distortum/duboisii/equinum/ferrugineum/gallinae/gypseum/nanum
Persicolor/praecox
Monosporium apiospermum
Nannizzia gypsea/obtussa/otae
Penicillium marneffei
Phialophora verrucosa
Pseudollescheria boydii
Rhinocladiella compacta/pedrosoi/spinifera
Rhinosporidium seeberi
Sporothrix schenckii
Trichophyton cocentricum/erinacei/equinum/gourvilli/megninii/mentagrophytes/rubrum
/schoenleinii/smii/soudanense/tonsurans/verrucosum/violaceum/yaoundei
Xylophora carrionii

FUNGI
Risk group III

Ajellomyces capsulatus/dermatitides
Coccidioides immitis
Histoplasma capsulatus (Ajellomyces capsulatus)
Histoplasma duboisii/farciminosum
Paracoccidioides brasiliensis
Zymonema dermatitides (Ajellomyces dermatitides)

VIRUSES
Risk group I

-attenuated viral strains which are accepted vaccines. Only a limited number of passages in defined cell-culture or host-systems are allowed
-apathogenic viral strains
-viral strains from fungal or bacterial systems, provided they do not contain virulence-factors and are described as apathogenic for higher animals and human beings
-Baculoviruses of insects
-apathogenic, endogeneous, animal retroviruses

VIRUSES
Risk group II

Adenovirus (provided they are not described in risk group 1)
Astro-virus
Aura-virus
Avian enzephalomycelitis-virus
Barnah forest virus
Bebaru virus
Bern-virus
BK-virus
Border disease virus
Borna virus
Bovine mucosal disease virus
Bovine ephemeral-fever virus
Breda virus
Bunyamwera virus

Chuzan virus
Colorado tick-fever virus
Corona virus
Coxsackie virus A and B
Cytomegalovirus
Ectromelia virus
exogenous retroviruses (i.e. murine mamma-tumor virus, feline immundefficiency virus)
Feline calcivirus
Fort morgan virus
Avian smallpox virus
Hepatitis A to E
Herpes simplex virus I and II
Human calcivirus
Human herpes virus 6
Human papilloma virus
Human rhinovirus
Human influenza virus type A, B, C
Shope fibrom virus
Cowpox virus
Lumpy skin disease virus
Minute virus in mice
Measles virus
Mumps virus
Murine penumoniae virus
Myxoma virus
Newcastle disease virus
Nyong-nyong virus
Orbi virus
Parainfluenza virus type 1-4
Parovirus
Horsepox virus
Pixuna virus
Polyomyelitis virus
Polyoma virus
Reo virus
Respiratory syncytial virus
Ross river virus
Rota virus
Sandfly virus
Semliki forest virus
Simian hemorrhagic virus
Stomatitis papulosa virus
Tanapox virus
Una virus
Uukumiemi virus
Vaccinia virus
Varicella virus

VIRUS
Risk group III

Monkeypox virus
Cabassou virus

Chikungunya virus
Dengue virus type 1-4
Eastern equine encephalitis virus
Enzephalitis virus
Epstein-Barr virus (cause of Kuru and Jacob-Creutzfeld disease)
Everglades virus
Yellow-fever virus
Hazara virus
Hepatitis B, virus
Hepatitis E virus
HIV 1 and 2
Kyasanur forest virus
Mayaro virus
Middleburg virus
Mucambo virus
Nairobi sheep disease virus
Oropuche virus
Papataci-fever virus
Powassan virus
Rabies
Rubella virus
SV40 virus

ANNEX- 5

FRAMEWORK FOR RISK ASSESSMENT

Framework to assess field testing of genetically modified plants:

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

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

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

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

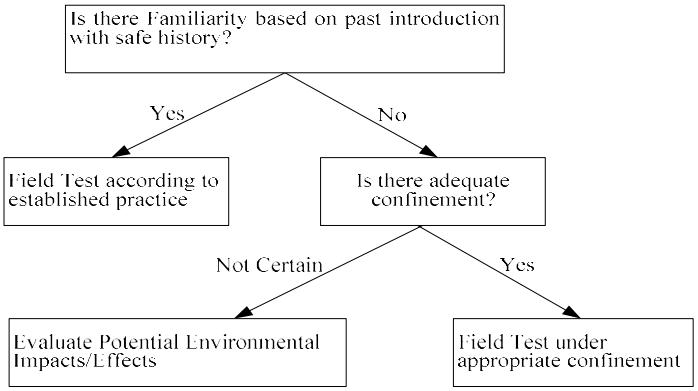

Fig-1. Framework to assess field testing of genetically modified plants

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

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

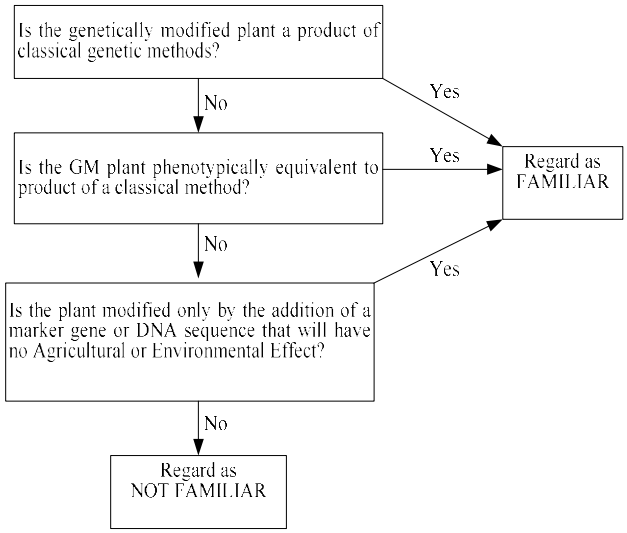

Fig- 2. Familiarity tests for genetically modified plants

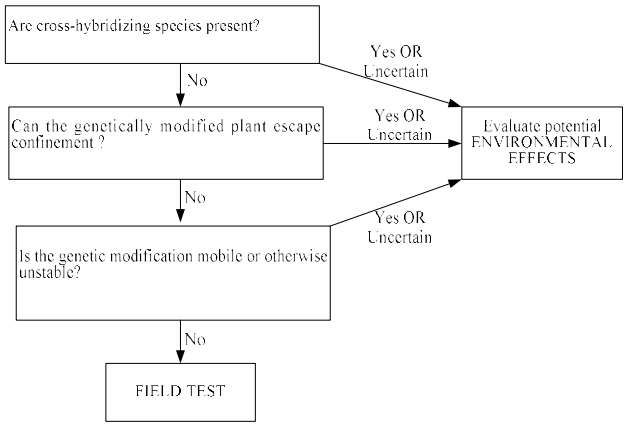

Fig-3. Confinement tests for genetically modified plants

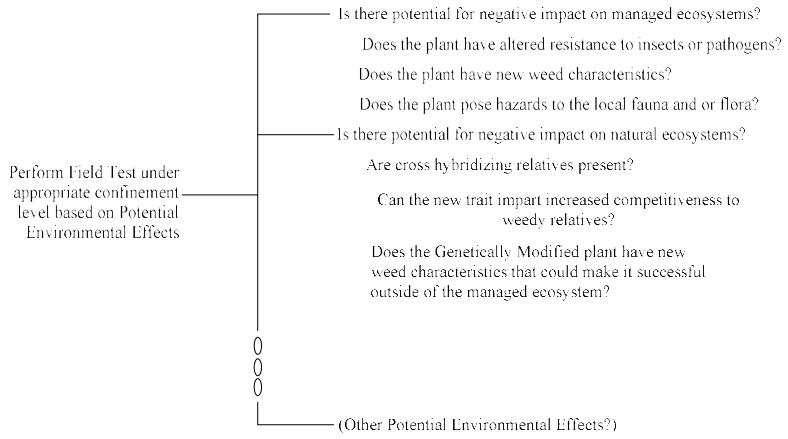

Fig-4. Potential environmental effects: appropriate question for specific applications to be added by users of the framework for release of genetically modified plants.

Framework to assess field of genetically modified microorganisms:

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

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

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

The next step after laboratory experimentation is to test modified microorganisms in the field, and establishing a scientifically based framework for decisions on field testing has been a primary purpose in this report. Not adverse effects of introductions have been seen and an extensive body of information documents safe introductions of some microorganisms, such as the rhizobia, mycorrhizal fungi, baculoviruses, Bacillus thuringiensis, and Agrobacterium. However, less is known about field tests of microorganisms than of plants. Thus, for unfamiliar applications, it is prudent to prepare for the control of the introduced microorganisms.

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

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

The framework neither distinguishes between classical and molecular methods of genetic manipulation, nor between modified and unmodified genotypes. The framework is product rather than on the methods by which it is obtained. Knowledge of the methods used may nonetheless yield useful information concerning the precision of genetic characterization of the microorganism, which in turn may be relevant for assessment of its similarity to previous applications persistence and possible effects after introduction.

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

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

In the framework, assessments of potential risks arising from the introduction of microorganisms into the environment are made according to the three major criteria of familiarity control and effects. Upon evaluation of these three criteria, a proposed introduction can be field-tested according to established practice or it can be assigned to one of three levels of concern: low, moderate or high uncertainty (Fig-5). The framework is inherently flexible, allowing an application to be reassigned to a different category as additional scientific information is obtained that is relevant to any of the three criteria.

Small-scale field tests can proceed according to established practice if the microorganism used its intended function and the target environment are all sufficiently similar to prior introductions that have a safe history of use (Fig-6). Rhizobium used for enhancement of nitrogen fixation in leguminous crops provides a familiar example.

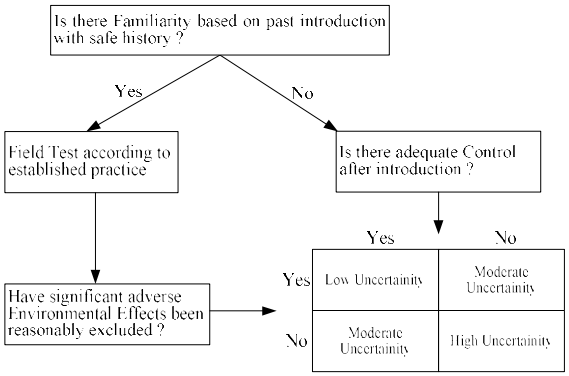

Fig-5. Framework to assess field testing of genetically modified microorganisms

If an introduction does not satisfy the familiarity criteria, it is evaluated with respect to both our ability to control the microorganism's persistence and dissemination and the microorganism's potential for significant adverse effects (Fig-5). For example, Rhizobium modified to encode an insecticidal toxin would not be a familiar introduction even though it might well prove to be safe. An introduction is considered to be in the low uncertainty category if it satisfies

appropriate criteria with respect to both controllability and low potential result in adverse effects. An introduction is considered to be in the moderate-uncertainty category if it satisfies criteria for either controllability or potential effects, but not both. An introduction is considered to be in the high uncertainty category if it satisfies neither the control nor the effects criterion (Fig-5). The high uncertainty status implies that potential adverse effects exist and are coupled with potential inability to control the microorganisms, and hence its potential effects.

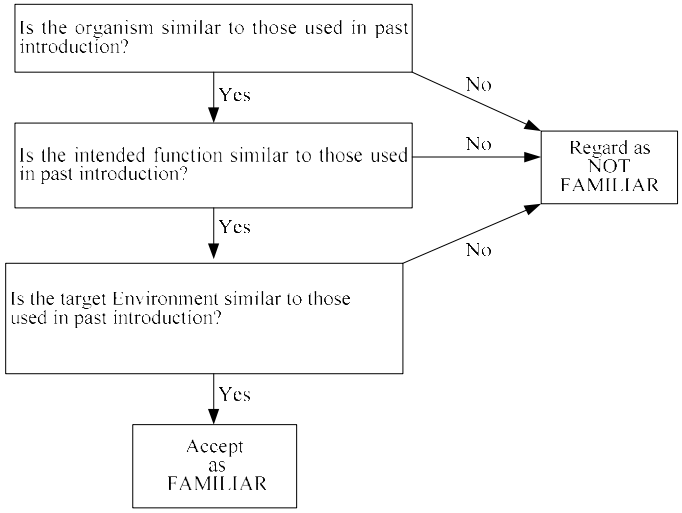

Fig-6. Familiarity tests for genetically modified microorganisms

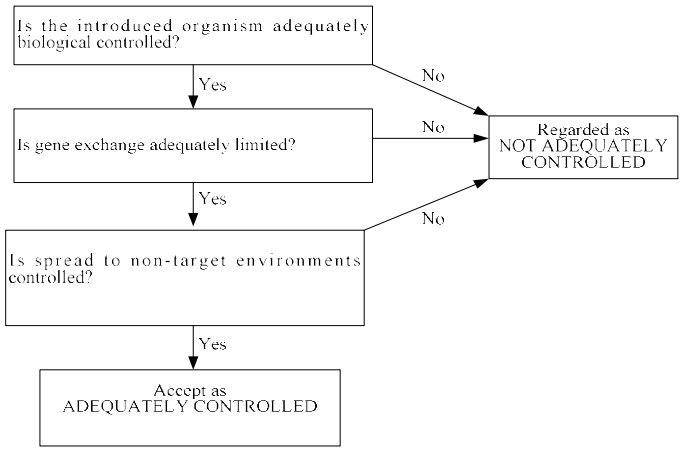

Fig-7. Appropriate question for specific applications to be added by users of the framework for the release of genetically modified microorganisms.

Specific criteria for evaluating control of the microorganism after it is introduced must include the potentials for persistence of the introduced microorganisms, genetic exchange between the introduced and indigenous microorganisms, and spread of the introduced microorganisms to non-target environments (Fig-7). A series of question

to be addressed in evaluating the potential for unwanted persistence of an introduced microorganism is illustrated in Fig-8.

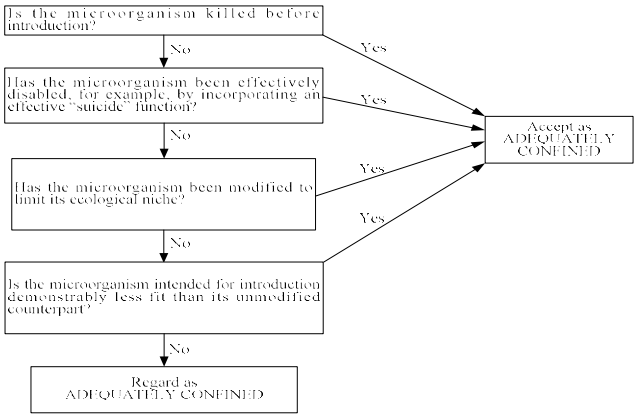

Fig-8. Biological confinement: Appropriate questions for specific applications to be added by users of the framework for the release of genetically modified microorganisms.

Criteria for evaluating effects must depend, at least in part, on the intended function of the introduced microorganism in its target environment (Fig. 9). Thus, a proposed field test of a bacterium to be used for biodegradation of a toxic pollutant should be preceded by definitive laboratory experiments and should be designed to determine whether toxic by-products of the degradation may be created and persist.

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

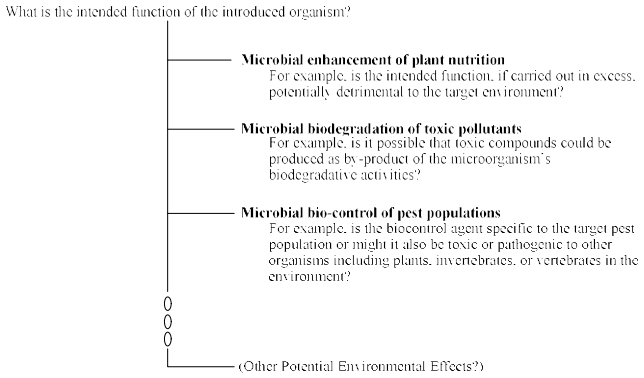

Fig-9. Potential Environmental Effects: Appropriate question for specific applications to be added by users of the framework for the release of genetically modified microorganisms.

ANNEX-6

INFORMATION NEEDED IN TRANS-BOUNDARY MOVEMENT OF GMO/LMO

Detailed information on the following matters should be included with the consignment of export or import of GMO/ LMO:

a. Name, address and contact details of the exporter.
b. Name, address and contact details of the importer.
c. Name and identity of the living modified organism, as well as the domestic classification, if any, of the Biosafety level of the living modified organism in the State of export.
d. Intended date or dates of the trans-boundary movement, if known.
e. Taxonomic status, common name, point of collection or acquisition, and characteristics of recipient organism or parental organisms related to biosafety.
f. Centres of origin and centres of genetic diversity, if known, of the recipient organism and/or the parental organisms and a description of the habitats where the organisms may persist or proliferate.
g. Taxonomic status, common name, point of collection or acquisition, and characteristics of the donor organism or organisms related to biosafety.
h. Description of the nucleic acid or the modification introduced, the technique used, and the resulting characteristics of the living modified organism.
i. Intended use of the living modified organism or products thereof, namely, processed materials that are of living modified organism origin, containing detectable novel combinations of replicable genetic material obtained through the use of modern biotechnology.
j. Quantity or volume of the living modified organism to be transferred.
k. A previous and existing risk assessment report consistent with Annex III.
l. Suggested methods for the safe handling, storage, transport and use, including packaging, labelling, documentation, disposal and contingency procedures, where appropriate.
m. Regulatory status of the living modified organism within the State of export (for example, whether it is prohibited in the State of export, whether there are other restrictions, or whether it has been approved for general release) and, if the living modified organism is banned in the State of export, the reason or reasons for the ban.
n. Result and purpose of any notification by the exporter to other States regarding the living modified organism to be transferred.
o. A declaration that the above-mentioned information is factually correct.

ANNEX- 7

INFORMATION REQUIRED CONCERNING LIVING MODIFIED ORGANISMS INTENDED FOR DIRECT USE AS FOOD OR FEED, OR FOR PROCESSING UNDER ARTICLE 11 OF CARTAGENA PROTOCOL ON BIOSAFETY

a. The name and contact details of the applicant for a decision for domestic use.
b. The name and contact details of the authority responsible for the decision.
c. Name and identity of the living modified organism.
d. Description of the gene modification, the technique used, and the resulting characteristics of the living modified organism.
e. Any unique identification of the living modified organism.
f. Taxonomic status, common name, point of collection or acquisition, and characteristics of recipient organism or parental organisms related to biosafety.
g. Centers of origin and centers of genetic diversity, if known, of the recipient organism and/or the parental organisms and a description of the habitats where the organisms may persist or proliferate.
h. Taxonomic status, common name, point of collection or acquisition, and characteristics of the donor organism or organisms related to biosafety.
i. Approved uses of the living modified organism.
j. A risk assessment report consistent with annexure III of the Cartagena Protocol.
k. Suggested methods for the safe handling, storage, transport and use, including packaging, labelling, documentation, disposal and contingency procedures, where appropriate.

ANNEX-8

BIOHAZARD COMMUNICATION

Biohazard communication and the Universal Biohazard Sign:

Biohazards should be communicated through labeling and biohazard signs. Where biologically active substances and wastes are used, handled or stored, labs should use the universal biohazard symbol. This symbol is required for bags, sharps containers, containers of contaminated laundry, refrigerators, and freezers used to store, transport or ship biologically active substances like rDNA or any hazardous microorganisms. In addition to labels, to post a biohazard sign at the entrance to a lab is utmost essential. The sign should include the universal biohazard symbol, the agent in use, the criteria for entry and the Biosafety level. Research labs also require the name and telephone number of a contact person; this is a good idea for all areas. The universal biohazard symbol is shown in Fig-10.

Fig-10: Universal Biohazard Sign

All lab employees should be adequately trained prior to beginning work with biologically active substances. Training should occur at the time of initial assignment and whenever changes in work tasks or operations create new exposure situations. Training should be tailored to the specific job. Proper management ensures that biologically active waste is properly handled from cradle to grave. The following elements of a biological waste management program should be in place to reduce exposure to employees and the public:

- Segregate infectious waste from the general trash;
- Use the universal biological hazard symbol on infectious waste containers;
- Select the packaging material that is appropriate for the type of waste handled:
    - Plastic bags for solid or semisolid infectious waste,
    - Puncture resistant containers for sharps, and
    - Bottles, flasks, or tanks for liquids;
- Use packaging that maintains its integrity during storage and transportation;
- Do not compact infectious waste or packaged infectious waste before treatment;
- Minimize storage time;
- Select the most appropriate treatment option for waste. Consider steam sterilization, incineration, thermal inactivation, and chemical disinfection.

Pollution Prevention and Biologically Active Substances

An effective biological waste program not only protects workers and the environment, it can also lead to cost savings from waste reduction or prevention. Lab staff and management should pursue opportunities to use materials with a lower biohazard level or alternative procedures to reduce the material handling and disposal requirements of the program.

Glossary of the Terms used in Biosafety Guidelines

For the purposes of this Guideline the following terms shall have the following meanings:

1. **Amino Acid -** Amino Acids are the molecular units of organic compounds that make up proteins.

2. **Anticodon-** a particular combination of three bases in transfer RNA (tRNA) that is complementary to a specific three-base codon in messenger RNA. Alignment of codons and anticodons is the basis for organizing amino acids into a specific sequence in a protein chain.

3. **Autecology-** That part of ecology which deals with individual species and their reactions to environmental factors.

4. **Bacterium-** one of a group of one-celled microorganisms having round, rodlike, spiral or filamentous bodies that are enclosed by a cell wall or membrane and which lack fully differentiated nuclei.

5. **Bases and Base-pairing rule -** Bases are essential building blocks of Deoxyribonucleic Acid (DNA) and Ribonucleic Acid (RNA). Human DNA consists of about 3 billion bases, and more than 99 percent of those bases are the same in all people. The order, or sequence, of these bases determines the information available for building and maintaining an organism, similar to the way in which letters of the alphabet appear in a certain order to form words and sentences. The information in DNA is stored as a code made up of four complementary chemical bases: adenine (A), guanine (G), cytosine (C), and thymine (T). DNA bases pair up with each other, A with T and C with G, to form units called base pairs. In RNA, the base uracil (U) replaces thymine and thus bases A pairs up with U and C pairs up with G. Each base is also attached to a sugar molecule and a phosphate molecule. Together, a base, a sugar, and a phosphate are called a nucleotide. Nucleotides are arranged in two long strands that form a spiral called a double helix. The structure of the double helix is somewhat like a ladder, with the base pairs forming the ladder's rungs and the sugar and phosphate molecules forming the vertical sidepieces of the ladder. Based on the sequence of bases in one strand of DNA, it is possible to predict the sequence in the opposite, complementary strands.

6. **Biohazard-** Hazard is an event or process that is potentially destructive to other physical or natural things or organisms. Biohazard is hazard to humans or the environment resulting from a living or biologically derived material or biological agents or conditions.

7. **Biosafety-** the policies and procedures adopted to ensure the environmentally safe application of biotechnology.

8. **Biotechnology-** any technique that uses living organisms or substances from these organisms to make or modify a product, to improve plants or animals, or to develop microorganisms for specific uses.

9. **Biological Safety Officer (BSO) :** Under Biosafety Guidelines there may be designated Biosafety Officers at the institute level who will be responsible for ensuring and implementing the issues of Biosafety at the institute level. According to Cartagena Protocol on Biosafety, the Officer/s under competent national authority who will be responsible for endorsing Biosafety related clearance in favor of the application of the proposals of import for contained use or commercial release of GMOs/LMOs.

10. **Cell-** the smallest component of life. A membrane-bound protoplasmic body capable o carrying on all essential life processes. A single cell unit is a complex collection of molecules with many different activities.

11. **Contained use-** any operation, undertaken within a facility, installation or other physical structure, which involves GMOs/LMOs that are controlled by specific measures that effectively limit their contact with, and their impact on, the external environment.

12. **Containment-** act of restricting or preventing the spread, leak or escape of an experimental object.

13. **Decontamination-** process of removing, destroying or reducing the activity of materials such as toxic chemicals, pathogenic microorganisms, etc. that could endanger an individual or the environment.

14. **DNA sequencing-** determination of the order of bases in a DNA molecule.

15. **Donor organisms-** the organism from which genetic material is obtained for transfer to the recipient organism.

16. **Environment-** humans and their surroundings including the earth's sub-surface.

17. **Enzyme-** a protein that accelerates a specific chemical reaction, without itself being destroyed.

18. **Export**- intentional trans-boundary movement from one country to another country.

19. **Exporter-** any legal or natural person, under the jurisdiction of a country of export, who arranges for a living modified organism to be exported.

20. **Gene-** the fundamental physical and functional unit of heredity, the portion of a DNA molecule that is made up of an ordered sequence of nucleotide base pairs that produce a specific product or has an assigned function.

21. **Genetic code-** the code that translates information contained in messenger RNA into amino acids. Different triplets of bases (called codons) code for each of 20 different amino acids.

22. **Genetic engineering-** technologies (including recombinant-DNA technologies) used to isolate genes from an organism, manipulate them in the laboratory, and insert them into another organism.

23. **Genotype-** the genetic constitution of an organism as distinguished from its physical appearance (phenotype)

24. **Germplasm**- the total genetic variability, represented by germ cells or seeds, available to a particular population of organisms.

25. **Genetically Modified Organism (GMO)-** a genetically-modified organism. These are living organisms whose genetic material has been altered or modified by any of the varieties of techniques of modern molecular biology to make them capable of producing news substances or perform new functions.

26. **GMO-Products-** the products involving Genetically Modified organisms (GMOs/LMOs) can be grouped into two (a) where GMOs/LMOs are used in the process of production but the end product is not GMO (the vaccine, growth hormones etc.) (b) where the end product is GMO (the plants with foreign genes with improved characteristics like resistance to insect, pests or virus etc.)

27. **Guidelines-** the Biosafety Guidelines, 2005.

28. **Host-vector (HV) system-** a microbial strain (host) and its compatible DNA carrier(s) (victor). The host may be a strain of the bacterium Escherichia coli or Bacillus subtilis, the yeast Saccharomyces cerevisiae or other such organisms that have been genetically manipulated to allow the multiplication and expression of the vector. The vector may be a plasmid, a bacteriophage or a virus, and other carriers of genetic materials all designed to carry readily selectable marker(s) and unique restriction sites for inserting DNA segments.

29. **Hybrid-** an offspring of a cross between two genetically unlike individual plants or animals.

30. **Hybridoma-** a new cell resulting from the fusion of a myeloma cell (a type of tumor cell that divides continuously in cultue) with a lymphocyte (an antibody-producing cell). Cultures of such cells are capable of continuous growth and specific (i.e. monoclonal) antibody production.

31. **Import-** intentional transboundary movement GMOs/LMOs into Bangladesh/one country from another country.

32. **Importer-** means any legal or natural person, under the jurisdiction of a country of import, who arranges for a living modified organism to be imported.

33. **Intellectual property-** that area of the law involving patents, copyrights trademarks, trade secrets and variety protection.

34. **Introduce (or introduction)-** to bring into or in-transit through Bangladesh to release into the environment, or to cause within land movement.

35. **Ligase-** an enzyme that joins the ends of DNA molecules together. These enzymes are essential tools in genetic engineering.
36. **Living Modified Organism (LMO)-** any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology. The term GMO or LMO are used interchangeably to denote the same thing pertaining to modern biotechnology.

37. **Living organism-** means any biological entity capable of transferring or replicating genetic material, including sterile organisms, viruses and viroids.

38. **Modern biotechnology-** the application of:
    a. In vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or
    b. Fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection.

39. **Monoclonal antibodies-** identical antibodies that recognize a single specific antigen and are produced by a clone of specialized cells.

40. **Move (moving, movement) –** to ship, offer for shipment, offer for entry, import, receive for transportation, carry, or otherwise transport or allow to be transported into, through, or within Bangladesh.

41. **Organism-** any active, infective, or dormant stage or life form of an entity characterized as living, including plants, bacteria, fungi, mycoplasmas, mycoplasma like entities, vertebrate and invertebrate animals, as well as entities such as viroids, viruses, or any living entity related thereto.

42. **Pathogen**- a disease-causing organism.

43. **Permit-** a written document issued by appropriate authority for the introduction of a regulated material under conditions that it will not present a risk of pest introduction/movement.

44. **Person-** any individual, partnership, corporation, company, society, association, or other organized group.

45. **Pest-** any living stage (including active and dormant forms) of insects, mites, nematodes slugs, 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 to humans, plant or animals or any processed, manufactured, or other products of plants or animals.

46. **Phage-** eating or destroying characteristic of a bacterial virus.

47. **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 parts (e.g. pollen, seeds, cells, tubers, stems) thereof and any cellular components (e.g. plasmids, ribosomes, etc.). Plant takes nutrient in soluble form.

48. **Plasmid-** a self-replicating, circular, extra-chromosomal DNA molecule.

49. **Product**- anything made by, or formed or derived from an organism, living, or dead.

50. **Recipient organism-** the organisms that receives genetic material from a donor organism.

51. **Recombinant DNA (rDNA) –** a DNA molecule into which a foreign DNA has been inserted.

52. **Regulated article or material –** any organism which has been, altered or produced through genetic engineering, if the donor organism, recipient organism, or vector or vector agent belongs to any genera or taxa and/which meets the definition of pest, or pathogen, or is an unclassified organism and/or an organism whose classification is unknown; or any product which contains such an organism or any other organism or product altered or produced through genetic engineering which the appropriate authority determines, or has reason to believe is a pest or pathogen. Excluded are microorganisms and products which are not pests or pathogens that have resulted from genetic manipulations in which all donor and recipient organisms and materials are well characterized and innocuous.

53. **Release into the environment-** the use of a regulated material outside the physical confinement found in a laboratory, a contained greenhouse, a fermenter or other contained structure.

54. **Responsible individual-** someone who has control and who will maintain control over the introduction of the regulated article and will assure that all conditions contained and requirements set in the permit are complied with. The responsible individual shall be a resident of the Bangladesh or may be a designate representative who is a resident of the Bangladesh.

55. **Restriction enzyme-** bacterial enzymes that recognize specific short sequences of DNA and cut the DNA where these sites occur.

56. **RELP-** restriction fragment length polymorphisms- fragments of differing lengths of DNA that distinguish individuals, produced by cutting with restriction enzymes. They result from variations in the DNA sequence and can be detected with radioactive probes and can be used as markers in breeding.

57. **RNA (ribonucleic aicd) –** nucleic acid and complementary to DNA- the three kinds of RNA important in the genetic process in cells are messenger RNA (mRNA), ribosomal RNA (rRNA) and transfer RNA (tRNA).

58. **Species-** reproductive communities and populations- that are distinguished by their collective manifestation of ranges of variations with respect to many different characteristics and qualities. Species is also a group of very similar organisms which breed and produce fertile offsprings. Different species may breed but do not produce fertile offsprings.

59. **Tissue culture-** the propagation of tissue removed from organisms in a laboratory environment that has strict sterility, temperature and nutrient requirements,

60. **Transboundary movement-** the movement of a genetically modified organism to or from Bangladesh/ one country to another country.

61. **Transcription –** the process of converting information in DNA into information contained in messenger RNA.

62. **Transfer RNA (tRNA) –** RNA that is used to position amino acids in the correct order during protein construction.

63. **Transformation-** introduction and assimilation of DNA from one organism to another via uptake of naked DNA.

64. **Transgenic animals or plants-** animals or plants whose hereditary DNA has been augmented by the addition of DNA from a source other than parental germ plasm, in a laboratory using recombinant DNA techniques.

65. **Translation-** the process of converting the information in messenger RNA into protein.

66. **Vector-** a carrier or transmission agent. In the context of recombinant-DNA technology, a vector in the DNA molecule used to introduce foreign DNA into host cells. Recombinant-DNA vectors include plasmids, bacteriophages, and other forms of DNA.

67. **Vector or vector agent-** organisms or objects used to transfer genetic material from the donor organism to the recipient organism.

ড. মোঃ নাসির উদ্দিন
উপ-সচিব