BIOSAFETY GUIDELINES FOR BANGLADESH

Ministry of Science and Technology
Government of the People's Republic of Bangladesh
1999

بسم الله الرحمن الرحيم

Lt. General Muhammad Noor Uddin Khan
psc (Rtd.), M.P
Minister
Ministry of Science & Technology
Government of the People's Republic of Bangladesh
Bangladesh Secretariate, Dhaka

Date

Message

Biotechnology is the cutting-edge of science. The tools of Biotechnology poised for both a challenge and tremendous opportunity for growth and development of mankind. The world is facing ever increasing and tremendous challenges of hyperbolic growth of population, environmental degradation including loss of Biodiversity at alarming rate, deforestation and desertification and depletion of natural resources.

With the development of biotechnology there arise great hope and aspiration to use this tools of Genetic Engineering & Biotechnology to boost the world food production aiming at food security as well as to enhance the quality of life through sustainable agriculture practice and sustainable use of other natural resources.

We need to tap the full potential of Biotechnology. To this end with the installation of National Institute of Biotechnology, one requisite has been fulfiled; however, there also exists a need for a regulation or guideline for safer handling of GMOs and smooth conduct of Biotechnological research. I am happy to note that Biosafety Guideline of Bangladesh has already been formulated. It will serve the purpose of the research scienctists, entrepreneurs and Industrialists who are interested in Biotechnology and its commercialised products.

I wish to thank all the experts, the institutions, and the Ministries which provided valuable inputs for the formulation of the guideline. I am confident that our scientists and entrepreneurs will come forward to make a headway in Biotechnological research and product development for the benefit of the vast improverished people of the land.

(Lt. General Muhammad Noor Uddin Khan)
psc.(Rtd.), M.P

M. Fazlur Rahman

Secretary

Telegram : Minscience
Telephone : 866144
Fax : 88-02-869606
E-mail : most @ bangla net
Ministry of Science & Technology
Government of the
People's Republic of Bangladesh
Building No. 6
Bangladesh Secretariat
Dhaka-1000

D.-O. No.

Date

Message

The second half of this century is the era of molecular biology as analogously the first half is the age of atomic physics. The dawn of the coming century will be embraced with the biotechnology revolution. The developed world due to their predominance in Science and Technology, already find ways to equip themselves with this tool which is evident from the multibillion dollar project of Human Genome. Paradoxically, the need of this untapped technology is more crucial, more pressing for the developing world in order to cope with ever increasing and culminated problems of malnutrition, scarcity of food and lack of health care.

Bangladesh is a least-developed country where man-land ratio is acutely adverse to the whole of ecosystem and natural resources. Obviously for us the need of the hour is sustainable use of natural resources as well as of the ecosystem. Challenges of the poverty, degradation of the environment and momentum of the population growth are interwoven and interlinked. In order to stop depletion of natural resources and to achieve eco-friendly environment we need to come out of the trap of abject poverty.

Biotechnology has a great prospect to alleviate poverty by increasing agricultural productivity and the development of new food sources through plant breeding, with simultaneous stimulation to other sectors. Bangladesh, being a tropical and riverine country, this tool has also great potential in exploring aquaculture, in conservation and in bioprospecting of our rich biodiversity.

It is well understood that biotechnological guideline is a prerequisite for initiating biotechnological research in a country in order to achieve the status of safe handling of GMOs and to protect environment from any anticipated adverse impact of GMOs. It is a matter of satisfaction that we have been able to prepare a Biosafety Guideline for Bangladesh which got the approval of highest authorities and entails the views of different stakeholders. We hope that with the adoption of this guideline a fundamental bottleneck in pursuing the biotechnological research has been removed. This event will be marked as a watershed in the field of biological research in Bangladesh. In this opportunity I would like to thank all the members of the task force and the workshop on formulation of Biosafety Guideline, who have made relentless efforts to accomplish this task. We also cherish that scientific community will now take more initiative in conducting biotechnological research and development for the socio-economic upliftment of the country.

21/12/1999

(M. Fazlur Rahman)

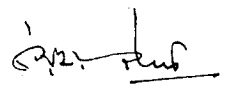
Foreword

Biotechnology has been exploited from time immemorial in different ways to serve various human needs. Modern Biotechnology and genetic engineering research have created tremendous momentum to present day research. By using genetic engineering technology several crop plant species have already been transformed with desired characters. For example. "flavr-savr" tomato, insect resistant varieties of cotton, corn, potato, herbicide tolerant varieties of soybean, cotton are already in the western market.

As biotechnological research may lead to various kinds of biohazards, internationally no laboratory is allowed to work in this line without having a proper biosafety measures. So, although Bangladesh is now capable of entering into this field of research yet it can not start without biosafety regulation; even the donor agencies are not able to help us without any guideline/regulation. It may be mentioned here that the development of appropriate biosafety guideline has become a priority for many governments since the "Rio Earth Summit" convention of biological diversity which entered into force in December 1993.

In the perspective of the above situation the Ministry of Science and Technology became keen on it and formed a taskforce for the preparation of Biosafety regulation in 1997 with Mr. Shah Nazmul Alam, Joint Secretary & Scientific Advisor, Ministry of Science & Technology as Chairman and we were given the responsibility to prepare the guideline. In this process the Bangladesh Association for Plant Tissue Culture was advised to organize a two-day workshop on Biosafety regulation with the concerned scientists, policy makers, UN bodies, NGO representatives etc. As an outcome of this venture with the assistance and support of the Ministry of Science & Technology, the workshop was held and the draft guideline was sent to concerned Ministries and a number of scientists of home and abroad to get their valuable comments. The draft was sent to the internationally reputed scientists in this field namely, Professor Roger Hull of John Innes Centre, Norwich, U.K. and Professor Hans Jorg Jacobsen of the University of Hannover, Germany. They have been very kind to go through the draft and sent their valuable suggestions. Their comments and suggestions have become very much helpful to finalize this document. Dr. Andre-de-Kathen of the University of Hannover has been very kind to be present in the workshop and played a key role in the preparation and finalization of this guideline.

Bangladesh now has become the member of the international Centre for Genetic Engineering and Biotechnology (ICGEB). As a result enormous opportunity has opened for us to be associated with world bodies in this field of research. We hope, this guideline will remain as a milestone in the field of Genetic Engineering & Biotechnology Research in Bangladesh.



Dr. Syed Hadiuzzaman
Professor, Department of Botany
University of Dhaka
and
Coordinator,
Workshop on Biosafety Guidelines

Dhaka
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WORKSHOP ON BIOSAFETY REGULATION

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IFST Auditorium, BCSIR, Dhaka

List of Resource Persons and Participants

Co-ordinator and Manuscript Preparation :

Professor Syed Hadiuzzaman, Director, Biotechnology Research Centre, University of Dhaka & General Secretary, Bangladesh Association for Plant Tissue Culture (BAPTC), Department of Botany, University of Dhaka.

Associate Co-ordinator :

Dr. M. Imdadul Hoque, Associate Professor, Department of Botany, University of Dhaka.

Resource Persons :

1. Dr. Naiyyum Chowdhury, Director, Planning & Development, AEC, Dhaka.
2. Professor Zeba Islam Seraj, Department of Biochemistry, University of Dhaka.
3. Professor R. H. Sarker, Department of Botany, University of Dhaka.

Foreign Expert :

Dr. Andre de Kathen, Institute of Molecular Genetics, University of Hannover, Herrenhauser Strasse 2, D-30419 Hannover, Germany.

Participants :

1. Professor M. M. Haque, Department of Botany, University of Dhaka.
2. Professor Mustafizur Rahman, Department of Biochemistry, University of Dhaka.
3. Professor Haseena Khan, Department of Biochemistry, University of Dhaka.
4. Mr. Bidyut Kanti Dutta, Associate Professor, Department of Pharmacy, University of Dhaka.
5. Professor M. Mozammel Haque, Department of Microbiology, University of Dhaka.
6. Professor Md. Nurul Amin, Department of Botany, University of Rajshahi.
7. Dr. M. Monzur Hossain, Department of Botany, University of Rajshahi.
8. Professor S. K. Bhadra, Department of Botany, University of Chittagong.
9. Dr. S. K. Roy, Department of Botany, Jahangirnagar University, Savar, Dhaka.
10. Dr. Golam Rabbani, Department of Horticulture, Bangladesh Agricultural University, Mymensingh.
11. Dr. A. B. M. Mamun Hossain, IFRB, Atomic Energy Research Establishment (AERE), Ganakbari, Savar, Dhaka.
12. Dr. Md. Al-Amin, Bangladesh Agricultural Research Institute (BARI), Gazipur.
13. Dr. Nilufar Hye Karim, Plant Physiology Division, BARI, Gazipur.
14. Dr. Mofazzal Hossain, Department of Horticulture, IPSA, Salna, Gazipur.
15. Dr. Tojammal Hossain, Biological Res. Division, BCSIR Lab., Mirpur Road, Dhaka.
16. Professor Gulroo Begum Sufi, Department of Zoology, University of Dhaka.
17. Dr. Asma Khatun, Breeding Division, Bangladesh Jute Research Institute (BJRI), Sher-e-Banglanagar, Dhaka.

18. Dr. Abdur Razzaque-II, Chief Scientific Officer (Crops), Bangladesh Agricultural Research Council (BARC), Farmgate, Dhaka.
19. Dr. S. A. Rashid, Planttech Ltd, 28, New Eskaton Road, Dhaka.
20. Dr. Abdur Razzaque Shah, Tissue Culture Division, BRAC, 75, Mohakhali, Dhaka.
21. Mr. Hassan Ali, Executive Director, IIRD, House 5/10, road No. 48, Block - E, Lalmatia, Dhaka.
22. Mr. M. Fazlur Rahman, Secretary, Ministry of Science & Technology, Government of the People's Republic of Bangladesh, Dhaka.
23. Mr. Shah Md. Nazmul Alam, Joint Secretary, Ministry of Science & Technology, Government of the People's Republic of Bangladesh, Dhaka.
24. Mr. Md. Abul Hashem Sarker, Deputy Secretary, Ministry of Science & Technology, Government of the People's Republic of Bangladesh, Dhaka.
25. Mr. Mihir Kanti Mozumder, Asstt. Scientific Adviser, Ministry of Science & Technology, Government of the People's Republic of Bangladesh, Dhaka.
26. Dr. A. J. M. Abdur Rouf, Sr. Assistant Secretary, Ministry of Science & Technology, Government of the People's Republic of Bangladesh, Dhaka.
27. Mr. Md. Mozibor Rahman, Deputy Secretary, Ministry of Science & Technology, Government of the People's Republic of Bangladesh, Dhaka.
28. Mr. Md. Khurshid-ul-Alam, General Manager, Banking Regulation & Policy Department, Bangladesh Bank, Motijheel, Dhaka.
29. Mr. Md. Sadique, Sr. Assistant Secretary, M.E.A.-1, Ministry of Foreign Affairs, Segun Bagicha, Dhaka.
30. Mr. Iqbal Kabir, Lawyer, Bangladesh Environment Lawyer Association (BELA), House No. 9, Road, No. 8, Dhanmondi R/A. Dhaka.
31. Mr. Chinmaya Mutsuddi, Bangladesh Environmental Journalists Forum, National Press Club, Dhaka.
32. Md. Mahmud Menon Khan, Research Associate, Bangladesh Centre for Development Journalism & Communication, 187, Green Road, Dhaka.
33. Dr. Harun-or-Rashid, Director, Bangladesh Medical Research Council, Mohakhali, Dhaka.
34. Mr. Shafi Ahmed, Laboratory Manager, Secretary to Director, ICDDR,B, Mohakhali, Dhaka.
35. Mr. Azaharul Majumder, Environmental Specialist, C/O. Mission Director, American Embassy, Baridhara, Dhaka.
36. Mr. Anwarul Islam, Country Head, IUCN - The World Conservation Union, House No. 13, Road No. 3, Dhanmondi R/A., Dhaka.
37. Dr. Md. Ali Ashraf, Programme Officer, UNDP, House No. 60, Road No. 11A (New), Dhanmondi R/A., Dhaka.
38. Dr. K. G. Pillai, Chief Technical Adviser, C/O. FAO Representative in Bangladesh, House No. 37, Road No. 8, Dhanmondi R/A., Dhaka.

Raporteurs

1. Professor Md. Abul Bashar, Department of Botany, University of Dhaka.
   Dhaka - 1000.
2. Dr. Sk. Shamimul Alam, Assistant Professor, Department of Botany, University of Dhaka.

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BIOSAFETY GUIDELINES FOR BANGLADESH

Introduction

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

Biotechnology consists of a gradient of technologies, ranging from the long established and widely used techniques of traditional biotechnology (e.g., food fermentation, biological control) through to modern biotechnology, which is based on the use of new techniques of recombinant-DNA technology (often called genetic engineering), monoclonal antibodies and new cell- and tissue culture methods.

The principal effect of the new techniques of modern biotechnology in agriculture is to broaden the range of hereditary material that can be utilized by conventional breeding programmes in the production of new varieties of useful plants, animals and microbes and their products. The applications developed from these new methods place them firmly within the continuum of techniques used in industry, agriculture and food processing throughout human history. Thus, while modern biotechnology provides powerful new tools, these tools are used to generate products that fill essentially the same roles as those produced with more traditional methods. The properties of these products do not differ substantially from those with which we are already familiar. By using genetic engineering technology several crop plant species have already been transformed with desired characters. For example "flavr-savr" tomato, insect resistant varieties of cotton, corn, potato, herbicide tolerant varieties of soybean, cotton, corn, etc. are already in the western market. Some other important transgenic lines are in the pipeline for release in the market.

Biosafety is used to describe the policies and procedures adopted to ensure the environmentally safe application of modern biotechnology. It is a term that is gaining wider currency as more countries benefit from the application of modern science in medicine, agriculture and the environment, without endangering public health or environmental safety.

In the application of modern biotechnology to medicine, agriculture or the environment, we are mainly dealing with organisms which are modified by use of either genetic engineering or other techniques. These can therefore, be referred to

as genetically modified organisms (GMOs) or hazardous organisms. The results of this modification need to be assessed for risk to humanity or the environment before the organism is freely released or deployed. The issues of public and environmental safety concerning every biotechnology product or its application must be carefully considered.

Scope of Guidelines

Biosafety guidelines are applicable for all research works - whether conducted in laboratories of the government research institutes, of state enterprises, universities, international organizations located in Bangladesh, of private companies and non-governmental organizations - involved in research and development activities of modern biotechnology. This guideline covers both laboratory and field trial of GMO's.

Objectives for introduction of Biosafety Guidelines in Bangladesh

The objectives of the biosafety guideline is to ensure safe transfer, handling and use of living modified organisms, specially focussing on safe guarding human and animal health, the environment, biological diversity and socio-economic welfare of the societies, including transboundary movement of any organisms.

Many research institutes in the country are engaged in modern biotechnological research. Skilled manpower is available for this purpose within the country. However at the moment collaborative projects with international research institutes are pending due to lack of proper biosafety guidelines. The government has also recognised the importance of biotechnological research in national development by showing its commitment for establishing a National Institute of Biotechnology. Adoption of biosafety guidelines is essential for any research institute working with modern biotechnology and genetic engineering to earn International recognition and to safely transfer the products of such research for public and environmental use.

Biosafety systems are intended to protect the entire environment of which biodiversity is a component. Under these circumstances the overall situation calls for a precautionary approach to ensure that Bangladesh is well prepared to manage biohazards. Hence it is advisable to :

(a) Develop guidelines, standards, codes of practice and monitoring capabilities for both research and development (R & D) and pre-release assessment of this risk associated with release into the environment of genetically modified organisms (GMOs) or hazardous organisms.

(b) Develop a sound scientific database, upon which risk assessment and evaluation of biotechnological products can be made.

(c) Provide guidelines to ensure safety of developers and end-users of biotechnology products.

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

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

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 judgement of safety or risk.

v. The primary responsibility for the safe use or transfer of organisms (hazardous or GMO's) 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 acceptor organism.

The assessment of the risk associated with modern biotechnological work can be subdivided into 5 areas depending on how and where GMO's or hazardous organisms or their products will be used. These areas are:

1. Work confined to the laboratory.
2. Use of GMO's or hazardous organisms in the field.
3. Direct release of foreign GMO's/hazardous organisms into our environment.
4. Industrial use of GMO's/hazardous organisms.
5. Products intended for release into the market.

Risk assessment criteria for Area 1 : Work confined to the laboratory

i. Principles of good laboratory practice (GLP) should be adhered to. GLP is concerned with the organisational process and the conditions under which laboratory studies are planned, performed, monitored, recorded and reported.

ii. Ensurance 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.

vi. Ensurance that health and safety precautions are applied according to national and/or international regulations.

v. Risk assessment should be dependant on and be according to the risk group for the GMO or hazardous organism being used.

Risk assesment criteria for Area 2 : Use of GMO's or hazardous organisms in the field

i. For field testing of GMO's 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 ease 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.

Risk assessment criteria for Area 3 : Direct release of foreign GMO's/hazardous organisms

i. GMO's or hazardous organisms which 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 are essential.

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

Framework for risk assessments for field test and release of GMO's is given in Annex 6 (prepared according to International Service for National Agricultural Research, ISNAR).

Risk assessment criteria for Area 4 : Industrial use of GMO's/hazardous organisms

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.

Risk assessment criteria for Area 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. Awareness of the fact that in a global context strategies for assessing the food safety and wholesomeness of these novel foods are still in a phase of exploration. Therefore risk assessment criteria may be subject to frequent change.

Risk management

For final release of a GMO, the authority should ensure that there is compliance with the safety conditions which are developed from the results of the risk assessment. It should include appropriate control procedure and procedures for terminating the experiment and disposing of wastes.

i. Risk assessment and management should be carried out by competent bodies. It may be necessary to seek expertise from outside the country. However, ultimate decisions must rest with the country.

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.

National and Institutional Biosafety Committees of Bangladesh

The National Committee on Biosafety of Bangladesh (NCBB) will be established to oversee the compliance with policies and guidelines in all institutions, public or private as well as co-ordinate with the appropriate national bodies that have regulatory powers over any violation. Institutional Biosafety Committees (IBC) will be established to create organizational awareness on biosafety and to ensure compliance with biosafety guidelines. The institution shall designate a working scientist as biosafety officer (BSO).

Risk management measures for controlled releases include

General precautions

* Appropriate information and training is provided for those involved in handling the organisms
* Monitoring procedures are applied in such a way that appropriate measures can be taken in case of unexpected effects during or after the release
* The dissemination of the released organisms and/or gene flow from the released organisms are controlled
* Controlling access to the release site

For plants

Applying reproductive isolation, by :

- 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

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

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
  - cell line should be less potentially carcinogenic
  - product (virus) should not carry oncogenic gene

These measures will often not be applicable once an organism with novel traits, such as a modified crop plant is at the stage of being marketed as a product if, as a result of testing during research and development it has been shown that the risks are acceptably low.

Activities not covered by this guidelines

The following activities are excluded from these guidelines :

(a) Work involving organisms within the country which result from natural reproduction or micropropagated products derived through tissue culture or the use of traditional breeding practices.

(b) Such other activities as the NCBB may in future declare to be excluded.

Sanctions against violation of biosafety guidelines

In addition to the revocation of the project approval, any violation of the provisions of this monograph or the concealment or withholding by the proponent of any information necessary to evaluate risks to human health or the environment shall be ground for the forfeiture of government research grants. Further, any incentives that may have been granted the proponent or institution for contributing to advanced scientific or technological research and development may be withheld. These penalties are exclusive of any other penalties that may be imposed under existing law, including, but not limited to, civil, criminal and administrative liabilities for gross negligence.

CHAPTER I

Biosafety Committees

In order to ensure safe management of biotechnology activities including research, development, introduction and use of GMO's, Bangladesh, like other countries need to establish different biosafety committees such as : A. National Committee on Biosafety of Bangladesh (NCBB), B. Institutional Biosafety Committee (IBC) and C. Field level Biosafety Committee (FBC). Accordingly the structure as well as responsibilities, power and functions of these committees have been defined in this chapter.

A. National Committee on Biosafety of Bangladesh (NCBB)

1. Composition of NCBB

1.1 The NCBB shall be composed of the following :

Chairperson : Secretary, Ministry of Science & Technology

Member Secretary : A Scientist (full time) with relevant experience from University/Scientific Organization to be appointed by the Ministry of Science and Technology.

Members :

(a) Scientists : to be selected by Ministry of Science and Technology

Bioscientists	6
Environmental Scientist	1
Medical Scientist (knowledgeable about human pathogens)	1

Bioscientists will include persons knowledgeable in biotechnology in the field of recombinant-DNA (rDNA), molecular biology, immunology, tissue culture, plant and animal breeding, microbiology, entomology, biochemistry, plant pathology and food science and nutrition.

(b) Representative of relevant ministries :

Will be designated by relevant authority from organisations under their jurisdiction .

Ministry of Science & Technology	1
Ministry of Environment & Forestry	1
Ministry of Agriculture	1
Ministry of Health	1
Ministry of Law and PA	1
Ministry of Industries	1
Ministry of Livestock and Fisheries	1
Ministry of Commerce	1

(c) Representative of farmers :

To be nominated by Ministry of Agriculture	1
(d) Representatives of FBCCI, MCCI, DCCI	2
(e) NGO representative (to be nominated by NGO bureau)	1
(f) Representative from Patent and Design	1
(g) A popular writer representing the general public to be nominated by the Ministry of Science and Technology	1

1.2. The tenure of members of the NCBB

1.2.1.The term of office of the Chairperson shall be co-terminus with his/her appointment as Secretary, Ministry of Science and Technology.

1.2.2. All members, excluding the Chairperson shall serve for a term of three (3) years, renewable for another term or more under exceptional circumstances provided that the members representing the committee shall hold the position for the duration of the term of their appointments in such agencies.

1.3. Qualifications of members of the NCBB

1.3.1. Bangladeshi citizens of unquestionable integrity who reside permanently in Bangladesh.

1.3.2. Qualification of member secretary : must have at least 10 years experience and having a Ph. D. degree in a relevant science.

1.3.3. The Scientist-members shall possess a minimum of 10 years experience in their respective fields.

2. Powers and functions of the NCBB

The NCBB shall have the following powers and functions.

2.1. Identify and evaluate potential risks in handling of hazardous or genetically modified organisms (GMO) and recommend measures to minimize risk. It has also to recommend measures to minimize risks which may result from the introduction of new species, strains or varieties. The NCBB can use tables in annex - 1 to determine the risk potential of organisms to be introduced.

2.2. The NCBB may call upon the experties (example: through e-mail) of an expatriate scientist for his/her views on identification and evaluation of potential risks in handling of hazardous or genetically modified organisms (GMO). It may also seek help in formulation, review or ammendment of national policies and guidlines on biosafety.

2.3. Formulate, review or amend national policies and guidelines on biosafety, risk assessment and risk management of work in biotechnology and supervise the implementation thereof.

2.4. Inform the government quarantine services at airport and border authorities on permission given on the import of GMOs and hazardous organisms.

2.5. Develop working arrangements with the government services, research institutions and universities, public health services in the evaluation, monitoring and review of work according to national policies and guidelines on biosafety. The NCBB reserves the right to inspect laboratories at any time without prior notice.

2.6. Recommend the development and promotion of research programmes to establish risk assessment protocols and assessment of long-term environmental effects of biological research covered by these guidelines. Preparation of different assessment forms e.g. project proposal forms for assessment of laboratory genetic manipulation work.

2.7. Publish the results of internal deliberations and agency reviews of the Committee.

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

2.9. Provide assistance in the formulation, amendment of pertinent laws, rules and regulations.

2.10. Call upon the assistance of experienced scientists from abroad if necessary or any agency, department, office, bureau including government-owned and/or controlled corporations.

2.11. Review the appointment of the members of the IBC upon recommendation of respective heads of institutions.

2.12. Hold discussions on the comparative ecological, economic and social impacts of alternative approaches to attain the purposes/objectives of the proposed genetic modification products and/or services.

2.13. Assess and identify priorities in human resource development in biosafety and in the related capacity building (including infrastructural) requirements.

2.14. The NCBB shall also update and modify the guidelines from time to time on the basis of scientific information and the experience gained on the use of new technique in the area of reserch and application.

2.15. Coordinate the activities of the technical committees.

3. Execution of the functions of NCBB

Member-Secretary will help execute the power and functions of NCBB

3.1. Member-Secretary

3.1.1. Will help execute the power and functions of NCBB.

3.1.2. Will receive all applications for permit, import and field release on behalf of Secretary of Science and Technology (Chairman, NCBB).

3.1.3. Will be responsible for documentation and preliminary screening of all applications. He will place suitable application to the notice of the Chairman, NCBB and to members of the NCBB prior to the bimonthly meetings.

3.1.4. Will be responsible for arranging the NCBB meetings and giving due notice to all the members.

3.1.5. Will maintain regular liaison through the institutional biosafety officer (BSO) with the various institutes and laboratories engaged in advanced biotechnological work.

3.1.6. Will be responsible for monitoring the use of biosafety guidelines by laboratories where BL-3 and BL-4 work is being undertaken and will pay irregular inspection visits.

3.1.7. The international scene in biosafety regulations is changing quite rapidly. The member-secretary will be assisted by the other members of the NCBB in keeping up with these changes by maintaining liaison with international bodies through internet and other communication media.

3.2. Scientific Officer

Will assist the member secretary of NCBB in execution of his functions. He should be recruited from the the biological sciences. May be on deputation or a government officer of the rank of assistant secretary or senior assistant secretary or having at least 3 - 5 years experience in relevant sciences.

3.3. Facilities to be provided to the Member-Secretary, NCBB

3.3.1. Transport facilities/TA/DA will be provided to the Member-Secretary and the scientific officer for activities related to the application of biosafety regulations.

3.3.2. Computer facilities with internet connections, a photocopying machine, fax and telephone will be provided for routine work.

4. Meetings of the NCBB

The committee shall meet quarterly and may hold special meetings to consider urgent matters at the decision of the Chairperson.

5. Quorum

$\frac{1}{3}$rd of the total scientist and $\frac{1}{4}$th of the other representative members shall constitute a quorum for the transaction of business.

6. Adoption of resolutions, guidelines or policies

The affirmative vote of at least half the scientist members and $\frac{1}{4}$th of the other representatives shall be necessary for the adoption of resolutions, guidelines or policies.

7. Honorarium to the NCBB members

The members shall be entitled to an appropriate per diem which shall be determined by the Ministry of Science and Technology.

8. Responsibilities and duties of NCBB

8.1. To examine within a specified time frame the application of any University department, division of a research institute or a private company and recommend to the Ministry of Science and Technology, whether or not permission can be given to the applying organization to do work on GMO's or hazardous organisms and their release provided that the department/division/company where the applicants intend to carry out their work have all biosafety measures available as specified and recommended in Chapter 2.

8.2. Should ensure implementation of biosafety measures for both on-site (field) and laboratory workers.

8.3. In case any local and/or foreign company applies for field testing or release of GMOs or hazardous organisms, NCBB must ensure that the applicant(s) adhere and strictly follow all biosafety measures as specified and recommended in Chapter 2.

8.4. The NCBB has to enforce that all institutes/Faculties/Companies already working with GMO's or hazardous organisms obtain the necessary permit within a year after adoption of the National Biosafety Guidelines.

8.5. No laboratory of the country should be involved in any research which are against the ethical matters (social or religious). The NCBB should regulate/monitor this aspect where ethical matters are involved.

B. The Institutional Biosafety Committee (IBC)

All institutions engaged in genetic engineering and potentially hazardous biological works are required to have an Institutional Biosafety Committee (IBC) which will evaluate and monitor the biosafety aspects of this 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 programme.

1. Composition of IBC

The head of the institution/department shall be the Chairperson of the IBC.

1.1. The committee has to be composed of 5 members from amongst staff members of institution and will be appointed by the Chairperson according to their expertise in genetic engineering or work with hazardous organisms.

1.2. IBC members may be entitled to an honourarium as determined by the Vice Chancellor/institutional head.

1.3. IBC may have consultants-on-call who are knowledgeable on the commitments and policies of the institution; applicable laws, standards of professional conduct and practice, community attitudes and the environment. Honourarium of the consultants on call shall be determined by the Vice Chancellor/institutional head.

2. Function of IBC

It shall be the function of IBC to :

2.1. Review work conducted at or sponsored by the institution and recommend research proposals for approval by the NCBB. The review shall cover :

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

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

2.1.3. Hold discussions on the comparative ecological, economic and social impacts of alternative approaches to attain the purposes/objectives of the proposed genetic engineering products/or services.

2.2. Notify the project chief/proponents/investigator about the results of the review.

2.3. Review at least once per year, the work with potential risks being conducted at the institution as well as review laboratory records on an irregular basis to ensure that requirements of the guidelines are being fulfilled.

2.4. Formulate and adopt emergency plans covering accidental spills and personnel contamination resulting from work.

2.5. Report immediately to the appropriate official in the concerned organization and to the NCBB any significant problems with the guidelines and any significant research-related accidents or illness.

2.6. Perform such other function as may be delegated by the NCBB and keep close liaison with NCBB.

3. **Meetings of the IBC**

The committee will normally meet every month and may hold special meetings to consider urgent matters upon the decision of the Chairperson.

4. **Quorum** :

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

C. The Biological Safety Officer(s) (BSO)

1. The BSO(s) shall be designated by the institution engaged in research, production and introduction activities involving genetic engineering and hazardous organisms. More than one BSO may be appointed depending on the needs of the institution.
2. It shall be the duty of the BSO to monitor compliance with the guidelines.
3. The BSO may or may not be a member of the IBC.
4. The BSO has to report regularly to the Chairman IBC, any matter regarding biosafety applications.
5. Honourarium to BSO : Honourarium to BSO shall be determind by the Vice Chancellor/head of the institute.

D. Field level Biosafety Committee (FBC)

Provisions for constitution of Field level Biosafety Committee (FBC) may be made in due course. NCBB will appoint three scientists and one representative from the Ministry of Environment to constitute the field level biosafety committee.

CHAPTER II

Procedures and guidelines on the introduction, movement and field release of regulated materials

Procedures and guidelines may vary according to the biosafety levels and the kind of application of GMO's or hazardous organisms. 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.

1. Procedures and guidelines for laboratories working with GMO's or hazardous organisms

1.1. Laboratories wanting to work with GMO's or hazardous organisms need permission from the Ministry of Science and Technology. 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 & 4. Once applications are made by a laboratory, permission needs to be given within 45 days. After permission is given, the specific laboratories will be registered accordingly (with respect to biosafety level) by the member secretary, NCBB.

1.2. Permission for each specific import for laboratory work at all levels of safety will not be required. The NCBB will only need to be informed about import of BL-2, BL-3 or BL-4 organisms. If required, the NCBB will inform customs at the port of entry about permission to import GMO's or hazardous organisms.

1.3. The written application for permission to work with GMO's or hazardous organisms should respond to all items listed (5.1 - 5.3) in the application procedure provided that these can be justifiably applied.

1.4. Laboratories permitted to work with GMO's or hazardous organisms have to follow the principles of Good laboratory practice (GLP) (see Annex 2).

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

2. Procedures and guidelines for field release of GMO's or hazardous organisms

2.1. Laboratories/companies/organizations seeking field test and release GMO's or hazardous organisms or use such organisms for large scale production will need to apply for a separate permit for each individual organism from NCBB through the IBC.

2.2. Application for a permit and a proposed procedure for release of GMO's or hazardous organisms into the environment duly endorsed by the IBC should be submitted to the NCBB 90 days in advance of the planned release. The written application should respond to all items listed in the application procedure (5.1 - 5.4). Non compliance of the provisions will call for action as prescribed in the rules.

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

2.4. All persons who are granted permit to release GMO's or hazardous organisms 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 (Annex - 3).

2.5. The NCBB together with local authorities 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.

2.5.1. Laboratories/companies/organisations permitted to field release GMO's or hazardous organisms have to maintain detailed records of experiments designed for the purpose of field release.

2.6. If any GMO or hazardous organism intended for field release needs to be imported, the NCBB will issue an import permit for each individual organism and inform the relevant authorities at the port of entry. The import permit will be separate from the permit to field release individual organisms.

2.7. NCBB 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. For institutions who have developed their own transgenic plants, within the country, these needed to be tested in an appropriate confined space (not necessarily or controlled

temperature glass house), where pollen escape can be prevented. The transgenic plants produced within the country should be maintained in a designated confined space especially prepared for this purpose. For example a green house can be made from a simple frame of metal poles covered with coarse mesh. The holes in the mesh can be about 2-3 mm. Plants can be grown in pots or in soil in large concrete beds. Transgenic plants will be planted in the centre and will be surrounded by several rows of non transgenic plants of the same species. The non transgenic plants will be surrounded by suitable plants which can act as pollen trap such as *Sesbania sesban*. Non transgenic plants can be monitored for incorporation of foreign gene from the transgenic plants through transfer of pollen. Conditions for above can be varied according to whether the transgenic plants species is self or cross pollinating.

2.8. NCBB through IBC has to ensure that a minimum isolation distance is maintained all around the transgenic plants and that a non compatible crop be grown in the isolation distance.

2.9. IBC will ensure that the seed progeny from the plants used as pollen trap be analyzed to assess the extent of pollen escape from the transgenic lines to compatible border row plants and to advise redesigning of the "Foundation seed" production in case of infiltration of pollen into border line plants.

2.10. IBC will ensure a minimum of two years field trials in small plots (away from physical and biological proximity to man and other significant biota) indicating stability and persistence of the character(s) of interest and other important agronomic characters as well as well-defined genetic markers in the released transgenic variety(ies).

2.11. IBC will ensure that the guideline in Annex 6 are followed for permission of micro-organism release to be given by the NCBB.

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

3. Procedures and Guideline for release of marketable products

3.1. Application for a permit for import and release of individual product has to be submitted to the NCBB 90 days in advance of the planned release. The application should respond to all items listed in the application procedure (5.1 - 5.4).

3.2. NCBB will grant a permit if warranted. In an application is denied/appropriate explanation will be given.

3.3. All organisations/companies who are granted permit to release products produced from GMO's or hazardous organisms into the market are required to keep records and submit periodic reports to NCBB regarding outcome of release or any allergic or adverse effect of released product.

3.4. All organisations/companies seeking a permit for release of products from GMO's or hazardous organisms 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.

4. Movement of regulated materials

4.1. No hazardous and other GMO's shall be introduced and moved unless specified guidelines on packaging and container requirements including marking and identification requirements are fully complied with.

4.2. All movements of hazardous organisms of GMO's shall include an advanced agreement between the participating laboratories and the IBC or BSO.

4.3. Any movement of regulated material will be accompanied by relevant permit issued by NCBB.

4.5. All the guidelines for movement shall be applied in the domestic transport within and between institutions.

5. Application procedure for permission to introduce, move or release GMO's or hazardous organisms or their products

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

5.1. Responsible person or persons involved

a. Name, title, address, telephone number and signature.

b. Name, address and telephone number of the person(s) who developed and/or supplied the regulated material.

5.2. Materials to be introduced

a. Quantity of the regulated material(s) to be introduced and proposed schedule and number of introductions.

b. All scientific and common designations necessary to identify the regulated materials.

c. Country and locality where the regulated material was collected, developed and produced.

d. Known potential to cause an epidemic (survival and reproductive rates, dispersal, etc.).

e. Classification of regulated matarial according to biosafety levels.

f. Known potential hosts or alternative hosts.

g. Known ability to evolve.

h. Known vector of organisms.

i. Known mode of spread and conditions for epidemic.

j. History of epidemics.

5.3. Genetically modified microorganisms

a. Nomenclature and detailed characteristics of donor, recipient and vector organisms.

b. 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.

c. 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 extrachromosomal), products and secretions and growth characteristics.

d. 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.

5.4. Additional requirement for field release/market

a. 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.

b. History of similar introductions.

c. A description of transfer of the regulated material (e.g., mail, common carrier, baggage or handcarried).

d. 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).

e. 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.

f. A detailed description of any biological material (e.g., culture medium or host material) accompanying the regulated material during movement.

g. A detailed description of the proposed method of final disposition of the regulated material.

h. Supervision of planned release.

i. Contingency plans to cope with extreme conditions (e.g. typhoon).

j. Consequences of the organism remaining in the environment beyond the planned periods.

k. Methods used (and their sensitivities) to monitor the planned release.

l. Methods used (and their efficiency) to control or eliminate the organism from the site and the surrounding environment, should circumstances so dictate.

m. Identification of the person who will undertake the release of the product in the field.

n. Other information :

- Information on the handler (not the broker).
- Knowledge of the handler on the specimen in question.

o. On-farm release of GMO's : On-farm environmental release procedure of GMO's should be developed in association/consultation with the Department of Environment. Advance information/notification should be given to the farmers/community of the test site of GMO's.

5.5. Assessment of subsequent behaviour of GMO or hazardous organism after field release

Subsequent hehaviour of GMO or hazardous organisms in the ecosystem after field release will be monitored by NCBB according to guidlines covered in section 5.4 & Annex 6. The time scale for monitoring of organism after field release will be determined by NCBB according to risk level of organism being introduced.

6. Issue of Permit

6.1. Permit will be issued according to guidelines specified for laboratory, field or market requirements as mentiond in sections 1, 2 & 3.

6.2. Any person whose application has been denied or whose permit has been withdrawn may appeal the decision 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 NCBB for final comments and suggestions.

6.3. 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.

6.4. 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.

CHAPTER III

Physico-chemical and Biological containments : Procedures and facilities

Since biotechnological research may be hazardous, 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.

1. Physical containment

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 the 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 compromize 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 judgement are key essentials 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 (BL1) 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.

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 is achieved through proper laboratory practices and containment equipment. Special laboratory design provides a secondary means of protection against the accidental release of 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 diferent 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 NCBB to other combinations that achieve an equivalent level of containment.

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.

1.3.1. Procedures

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

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

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

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

1.3.1.5. 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.

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

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

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

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

1.3.2. Containment equipment

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

1.3.3. Laboratory Facilities

1.3.3.1. The laboratory is designed so that it can be easily cleaned.

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

1.3.3.3. Laboratory furniture is sturdy. Spaces between benches, cabinets and equipment are accessible for cleaning.

1.3.3.4. Each laboratory contains a sink for handwashing.

1.3.3.5. If the laboratory has windows that open, they are fitted with fly screens;

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

1.4. Biosafty 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.

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

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

1.4.1.2. 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.

1.4.1.3. 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.

1.4.1.4. 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 5) 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 programme is in effect as certified by a licensed pest control operator.

1.4.1.5. 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.

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

1.4.1.7. 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.

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

1.4.1.9. Hypodermic needles and syringe are used only for parenteral 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.

1.4.1.10. 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.

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

1.4.1.12. 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.

1.4.2. Containment equipment

1.4.2.1 Biological safety cabinets (see Section 4, Class I or II) or other appropriate personal protective or physical containment devices are used whenever necessary.

1.4.2.2. Proper containment is applied when pocedures 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 intra-nasally, and harvesting infected tissues from animals or eggs.

1.4.2.3. 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.

1.4.3. Laboratory Facilities : All facilities under BL1 from 1.3.1.1 to 1.3.1.9 are applicable. In addition the following facility should be present.

1.4.3.1. An autoclave for decontaminating laboratory wastes is available.

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.

1.5.1. Procedures : All procedures under BL1 (1.3.1.1 - 1.3.1.9) and BL2 (1.4.1.1 - 1.4.1.12) are applicable. In addition the following procedure have to be followed for BL3.

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

1.5.1.2. Persons under 18 years of age are not allowed to enter the laboratory.

1.5.1.3. 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 pratices.

1.5.1.4. Laboratory doors are kept closed when experiments are in progress.

1.5.1.5. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable, leakproof container, which is closed before being removed from the laboratory.

1.5.1.6. The laboratory director controls access to the laboratory and restricts access to persons whose presence are required for programme or

support purposes. The director has the final responsibility of assessing each circumstance and determining who may enter or work in the laboratory.

1.5.1.7. The laboratory director establishes policies and procedures whereby only persons who have been advised 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 or animal rooms.

1.5.1.8. 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.

1.5.1.9. 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.

1.5.1.10. 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 programme is in effect as certified by a licensed pest control operator.

1.5.1.11. 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.

1.5.1.12. 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.

1.5.1.13. Molded surgical masks or respirators are worn in rooms containing experimental animals.

1.5.1.14. Animals and plants not related to the work being conducted are not permitted in the laboratory.

1.5.1.15. 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.).

1.5.1.16. All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

1.5.1.17. Vacuum lines are protected with High Efficiency Particulate Air (HEPA) filters and liquid disinfectant traps.

1.5.1.18. 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 the function of the laboratory.

1.5.1.19. 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.

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 aerosol 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.

1.5.3. Laboratory facilities

1.5.3.1. 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-doored 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.

1.5.3.2. 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.

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

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

1.5.3.5. 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.

1.5.3.6. Windows in the laboratory are closed and sealed.

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

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

1.5.3.9. 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 recirculated to any other area of

the building, is discharged to the outside, and is dispersed away from 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.

1.5.3.10. The HEPA-filtered exhaust air from Class I or Class II biological safety cabinets may be recirculated 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 exhaust system.

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.

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

1.6.1.1. 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.

1.6.1.2. 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.

1.6.1.3. Only persons whose presence are required for programme 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, biohazards 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.

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

1.6.1.5. 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, is 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 hamper in the inner change room.

1.6.1.6. 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 5) 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).

1.6.1.7. Supplies and materials needed in the faciltiy are brought in through the double-doored 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 or the autoclave, fumigation chamber, or air lock. These doors are secured after materials are brought into the facility. An insect and rodent control programme is in effect as certified by a licensed pest control operator for all levels.

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

1.6.1.9. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is intergral 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, cannulas are used instead of sharp needles.

1.6.1.10. 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.

1.6.1.11. 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.

1.6.1.12. 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.

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.

1.6.3 Laboratory facilities

1.6.3.1. 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-doored autoclave, fumigation chamber, or ventilated air lock is provided for passage of materials, supplies, or equipment that are not brought into the facility through the change room.

1.6.3.2. 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.

1.6.3.3. 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.

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

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

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

1.6.3.7. 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.

1.6.3.8. 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.

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

1.6.3.10. A double-doored 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.

1.6.3.11. 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.

1.6.3.12. 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 indicator microorganisms.

1.6.3.13. An individual supply and exhaust air ventilation system is provided. The system maintains pressure differentials and directional airflow as required to assure flows inward from areas 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 to assure inward (or zero) airflow at all times.

1.6.3.14. 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.

1.6.3.15. The treated exhaust air from Class I and 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 faciltiy 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.

1.6.3.16. A specially designed suit area may be provided in the faciltiy. 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-doored autoclave is provided for decontaminating waste materials to be removed from the suit area.

2. Biological containment

2.1. In considering biological containment, the vector (palsmid, organelle, or virus) for the rDNA and the host (bacterial, 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 : (i) survival of the vector in its host outside the laboratory and (ii) 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 :

2.1.1. 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.

2.1.2. HV2. These are host vector systems shown to provide a high level or biological containment as demonstrated by data from suitable tests 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 (i) as a prophage (in the inserted or plasmid form) in the laboratory host used for phage propagation or (ii) by surviving in natural environments and transferring a cloned DNA fragment to other hosts (or their resident prophages).

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

2.3. Certification of host-vector systems

2.3.1. Responsibility. HV1 systems other than *E. coli* K-1 and HV2 host-vector systems may not be designated as such until they have been certified by the chairperson of the NCBB. Application for certification of a host-vector system should be written and adressed to :

The Chairperson
National Committee on Biosafety of Bangladesh (NCBB).

2.3.2. Host-vector systems that are proposed for certification will be reviewed by the NCBB. 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 NCBB and other persons chosen because of their expertise in evaluating such data. The NCBB will then evaluate the report of the working group and any other available information at a regular meeting.

The Chairperson of the NCBB 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 propertis relevant to containment, may be certified by the NCBB Chairperson.

2.3.3. When a new host-vector system is certified, NCBB 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 NCBB at any time.

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

2.3.5. 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 NCBB 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.

2.4. Data to be submitted for certification

2.4.1. 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 organisms 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.

2.4.2. HV2 Systems. Investigators planning to request HV2 certification for host-vector systems can obtain instructions from NCBB 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 enviornment; (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 transfection 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 NCBB. 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 NCBB and its subcommittee.

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

This part of the Guidelines specifies physical containment guidelines 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 :

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

3.1. Selecting physical containment levels

The selection of the physical containment level required for rDNA research or production involving more than 10 litres 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 moelcules that require BL1 containment at the laboratory scale. The BL2-LS 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 BL3 containment at the laboratory scale.

No provisions are made 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 NCBB on an individual basis.

3.1.1. BL1-LS Level

3.1.1.1. 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.

3.1.1.2. 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.

3.1.1.3. 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.

3.1.1.4. 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.

3.1.1.5. 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.

3.1.1.6. 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.

3.1.2. BL2-LS Level

3.1.2.1. 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.

3.1.2.2. 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.

3.1.2.3. 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.

3.1.2.4. 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.

3.1.2.5. 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.

3.1.2.6. 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.

3.1.2.7. 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.

3.1.2.8. 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.

3.1.2.9. A closed system used for propagating and growing viable organisms containing rDNA molecules shall be permanently identified. This identification shall be used in all records 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.

3.1.2.10. 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.

3.1.2.11. 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.

3.1.3. BL3-LS Level

3.1.3.1. 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.

3.1.3.2. 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.

3.1.3.3. 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.

3.1.3.4. 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.

3.1.3.5. 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.

3.1.3.6. 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.

3.1.3.7. 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.

3.1.3.8. 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.

3.1.3.9. 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.

3.1.3.10. 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 records 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.

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

3.1.3.12. 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.

3.1.3.13. 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-doored 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 an 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 recirculated 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.

3.1.3.14. The following personnel and operational practices shall be required:

a. Personnel entry into the controlled area shall be through the entry area specified in Section 3.1.3.13a.

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 programme 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 programme shall be maintained.

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

4.1. 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.

4.2. 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.

4.3. 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.

5. Container requirements

5.1. 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.

5.2. 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.

5.3. Live microorganisms and/or etiologic agents, cells, or sub-cellular elements. All regulated materials which are live (non-inactivated) microorganisms or etiologic agents, cells or sub-cellular elements shall be packed as specified below:

5.3.1. 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). Several primary containers may be enclosed in a single 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 container(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.

5.3.2. Volume exceeding 50 ml

Regulated materials 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 or 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.

5.4. 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.

5.5. 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 sampels, 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 microoganisms 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 infectious agent and the target organism. Furthermore, the resistance or susceptibility of a microorganisms to adverse conditions such as temperature, pH, moisture and the like influence the chances of possible infections.

Table 1. Classification of infective micriorganisms by risk groups requirement of biosafety levels (WHO, Laboratory biosafety manual, 1983).

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 Gruop 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 ot anoter.

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 microoranisms 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 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 environhment. Prophylactics are available and treatment is effective.

Class III : Medium Risk

This class contains those microorganisms that offer severe threat ot the health of laboratory workers but comparatively small risk to the population at large. Prophylactics are available and treatement is effeective.

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 gerneral effective prophylactices are not available and no effecitive 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).

From Former and Kramer (1990)

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 immunodeficient 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 concentraions without offering any threat to normal persons (Collins 1990).

Meidum 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, ther is a strong tendency to replace medium- or high-risk operations by harmless techniques using the possibilites offered by the methodology of genetic engineering; the well known exmaple 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 organisms to be transfered 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 greately 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 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 pathogenes 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 pathogenes 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 Formmer and Kramer (1990)

Good Microbiological Techniques (GMT). A summary of the GMT is presented in Table 4.

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. Nervertheless, it is now generally accepted that genetically modified or engineered organisms must not at all be regarded as hazardous *per se*, but can be classifed 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.

Table 4. Good Microbiological Techniques (GMT) for the safe handling of microorganisms with risk potential.

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

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 no 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 transfereed accordingly to work with recombinant-DNA organisms.

This general statement has been broadly accepted worldwide, also in those countries in which genetic engineerring is regulated 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 repoduces the OECD recommendations of 1986 literally, specifies the following principles for working with group I 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 parenthesies):

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 programme 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 organisation and personnel

1.1. Management's respondibilities

1. Test facility management should ensure that the Principles of Good Laboratory Practice are complied within the test facility

2. **At a minimum it should**

a) ensure that qualified personnel, appropriate facilities, equipment and materials are available.

b) maintain a record of the qualifications, training, experience and job description for each professional and technical individual;

c) ensure that personnel clearly understand the functions they are to perform and, where necessary, provide training for these functions;

d) ensure that health and safety precautions are applied according to national and/or international regulations;

e) ensure that appropriate Standard Operating Procedures are established and followed;

f) ensure that there is a Quality Assurance Programme with designated personnel;

g) where appropriate, agree to the study plan in conjunction with the sponsor;

h) ensure that amendments to the study plan are agreed upon and documented;

i) maintain copies of all study plans;

j) maintain a historical file of all Standard Operating Procedures;

k) for each study ensure that a sufficient number of personnel is available for its timely and proper conduct;

l) 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;

m) ensure that an individual is identified as responsible for the management of the archives;

1.2. Study Director's responsibilities

1. The Study Director has the responsibility for the overall conduct of the study and for its report.
2. These responsibilities should include, but not be limited to, the following functions:

a) should agree to the study plan;

b) ensure that the procedures specified in the study plan are followed and that authorisation for any modification is obtained and documented together with the reasons for them;

c) ensure that all data generated are fully documented and recorded;

d) 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;

e) 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

1. Personnel should excercise safe working practice. Chemicals should be handled with suitable caution until their hazard(s) has been established. Special precaution should be excircised by pregnent women.
2. Personnel should exercise health precautions to minimise risk to themselves and to ensure the integrity of the study.

3. 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 programme

2.1 General

1. The test facility should have a documented quality assurance programme to ensure that studies performed are in compliance with these Principles of Good Laboratory Practice.
2. The quality assurance programme 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.
3. This individual(s) should not be involved in the conduct of study being assured.
4. 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

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

a) ascertain that the study plan and Standard Operating Procedures are available to personnel conducting the study;

b) 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;

c) promptly report to management and the Study Director unauthorised deviations from the study plan and from Standard Operating Procedures;

d) 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;

e) prepare and sign a statement, to be included with the 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

1. The test facility should be of suitable size, construction and location to meet the requirements of the study and minimise disturbances that would interfere with the validity of the study.
2. 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.

3.2. Test system facilities

1. The test facility should have a sufficient number of rooms or areas to assure the isolation of test systems and the isolation of individual projects, involving substances known or suspected of being biohazardous.
2. Suitable facilties should be available for the diagnosis, treatment and control of diseases, in order to ensure that there is no unacceptable degree of deterioration of test systems.
3. There should be storage areas as needed for supplies and equipment. Storage areas should be separated from areas housing the test systems and should be adequately protected against infestation and contamination. Refrigeration should be provided for perishable commodities.

3.3 Facilities for handling test and reference substances

1. To prevent contamination or mix-ups, there should be separate areas for receipt and storage of the test and references and mixing of the test substance with a vehicle.
2. Storage areas for the test substances should be separate from areas housing the test systems and should be adequate to preserve identity, concentration, purity and stability and ensure safe storage for hazardous substances.

3.4 Archive facilities

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

3.5 Waste disposal

1. Handling and disposal of wastes should be carried out in such a way as not to jeopardise the integrity of studies in progress.
2. 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

1. 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.
2. 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

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

4.3. Reagents

1. Reagents should be labelled, 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

1. Apparatus used for the generation of physical/chemical data should be suitably located and of appropriate design and adequate capacity.
2. Reference substances should be used to assist in ensuring the integrity of the physical/chemical test systems.

5.2. Biological

1. 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.
2. 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.
3. 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.
4. Records of source, date of arrival and arrival condition should be maintained.
5. Animal, plant, microbial and cellular test systems should be acclimatised to the test environment for an adequate period before a study is initiated.
6. All information needed to properly identify the test systems should appear on their housing or containers.
7. 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

1. Records including substance characterization, date of receipt, quantities received and used in studies should be maintained.
2. 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.
3. Storage container(s) should carry identification information, earliest expiration date and specific storage instructions.

6.2 Characterization

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

2. 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.
3. The stability of test and reference substances under conditions of storage should be known for all studies.
4. The stability of test and reference substances under the test conditions should be known for all studies.
5. 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.
6. 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

1. 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.
2. 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

1. 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, labelling, 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 computerised 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

1. For each study, a plan should exist in a written form prior to initiation of the study.
2. The study plan should be retained as raw data.
3. 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 :

1. Identification of the study, the Test and Reference Substance;
   a) A descriptive title;
   b) A statement which reveals the nature and purpose of the study;
   c) Identification of the test substance by code or name (IUPAC, CAS number etc);
   d) The reference substance to be used.
2. Information Concerning the Sponsor and the Test Facility
   a) Name and address of the Sponsor;
   b) Name and address of the Test Facility;
   c) Name and address of the Study Director.
3. Dates
   a) 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;
   b) The proposed starting and completion dates.
4. Test Methods
   a) Reference to OECD Test Guideline or other test guideline to be used.
5. Issues (where applicable)
   a) The justification for selection of the test system;
   b) Characterization of the test system, such as the species, strain, substrain, source of supply, number, body weight range, sex, age, and other pertinent information;
   c) The method of administration and the reason for its choice;
   d) The dose levels and/or concentration(s), frequency, duration of administration;
   e) 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.

6. Records

a) A list of records to be retained.

8.3. Conduct of the study

1. A unique identification should be given to each study. All items concerning this study should carry this identification.
2. The study should be conducted in accordance with the study plan.
3. 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 initialled and dated.
4. 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.
5. 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

1. A final report should be prepared for the study.
2. The use of the International System of Units (SI) is recommended.
3. The final report should be signed and dated by the Study Director.
4. If reports of principal scientists from co-operating disciplines are included in the final report, they should sign and date them.
5. Corrections and additions to a final report should be in the form of an amendment. The amendment should clearly specify the reason 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 :

1. Identification of the Study, the Test and Reference Substance
   a) A descriptive title.
   b) Identification of the test substance by code or name (IUPAC, CAS number, etc.);
   c) Identification of the reference substance by chemical name;
   d) Characterization of the test substance including purity, stability and homogeneity.
2. Information Concerning the Test Facility
   a) Name and address;
   b) Name of the Study Director
   c) Name of the principal personnel having contributed reports to the final report.
3. Duties
   a) Dates on which the study was initiated and completed.
4. Statement
   a) A Quality Assurance statement certifying the dates inspections were made and the dates any findings were reported to management and to the Study Director.
5. Description of Materials and Test Methods
   a) Description of methods and materials used.
   b) Reference to OECD Test Guidelines or other test guidelines.
6. Results
   a) A summary of results;
   b) All information and data required in the study plan;
   c) A presentation of the results, including calculations and statistical methods;
   d) An evaluation and discussion of the results and, where appropriate, conclusions.
7. Storage
   a) 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

1. Archives should be designed and equipped for the accommodation and the secure storage of :

   a) The study plans.

   b) The raw data.

   c) The final reports.

   d) The reports of laboratory inspections and study audits performed according to the Quality Assurance Programme.

   e) Sample and specimens.

2. Materials retained in the archives should be indexed so as to facilitate orderly storage and rapid retrieval.

3. Only personnel authorised by management should have access to the archives. Movement of material in and out of the archives should be properly recorded.

10.2. Retention

1. The following should be retained for the period specified by the appropriate authorities :

   a) The study plan, raw data, samples, specimens, and the final report of each study;

   b) Records of all inspections and audits performed by the Quality Assurance Programme;

   c) Summary of qualifications, training, experience and job descriptions of personnel;

   d) Records and reports of the maintenance and calibration of equipment;

   e) The historical file of Standard Operation Procedures.

2. Samples and specimens should be retained only as long as the quality of the preparation permits evaluation.

3. 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 sugested to classify a genetically modified organisms into the lowest risk group (Tables 5 & 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 5. Criteria for classifying genetically modified microorganisms in Group I.

A. Recipient or Parenteral Organism

- Non-pathogenic
- No adventitious agents
- 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 environment.

B. Vector/Insert

- Well characterized and free from known harmful sequences
- Limited in size as much as possible to the genetic sequences required to perform the intended function
- Should not increase the stability of the construct in the environment (unless that is a requirement of intended function)
- Should be poorly mobilizable
- 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

- Non-pathogenic
- As safe in the reactor or fermentor as recipient or 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.

- Those constructed entirely from a single prokaryotic recipient (including its indigenous plasmids and viruses) or from a single eukaryotic recipient (including its chloroplasts, mitochondria, plasmids, but excluding viruses)
- Those that consist entirely of genetic sequences from different species that exchange these sequences by known physiological processes

Table 6. Relationship between risk class, containment category and objectives of safety precautions.

Risk class	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 contaminantion of culture or product.
Class 2	C 1	Minimize the release of low-risk microorganisms from primary containment, no secondary containment.
Class 3	C 2	Prevent release of medium-risk microorganisms from primary containment during regular operations; no strict secondary containment.
Class 4	C 3	Absolute containment for high-risk microorganisms; secondary containment strictly required to prevent release in case of breach of primary containment.

Table 7. Relationship between risk class, containment category and safety precautions.

Harmless microorganisms (Class 1)

GILSP

Good occupational safety and hygiene principles are to be applied

Low risk microorganisms (Class 2)

Containment category 1

- GMT are to be applied
- Surfaces within the facility should be easily cleaned and disinfected
- Contaminated materials are to be autoclaved or disinfected before cleaninng
- Aerosol creating procedures must be controlled and contained
- Access to the facility must be restricted

Medium risk microorganisms (Class 3)

Containment category 2

Measure in addition to containment 1 conditions :

- Only authorized personnel is admitted to the facility

(Contd.)

(Contd.)

- 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

High Risk Microorganisms (Class 4)

Containment category 3

Measures in addition to containment 2 conditions :

- No visitors should be admitted
- Facility must be completely isolated
- The rooms 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 8. Safety precautions for biotechnological operations.

Measures, Operations, Equipment Design, Facilities	GILSP	Containment category		
		1	2	3
Procedure				
Written instructions and code of practice	+	+	+	+
Biosafety manual	–	+	+	+
Good occupational hygiene	+	+	+	+
Good microbiological techniques	–	+	+	+
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 constainment : Facilities				
Protective clothing according to risk category	+	+	+	+
Changing/washing facility	+	+	+	+
Disinfection 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.

4.4. Classification of safety measures

4.4.1. The 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 & 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 9. 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 be 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 so as to:	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

(Contd.)

(Contd.)

Containment measures	Containment levels		
	2	3	4
a) Biohazard signs should be posted	Optional	Yes	Yes
b) Access should be restricted to nominated personnel only	Optional	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 applications of biotechnology are imperative since possible risk in research and devolopment involving different micro organisms have been recognized. According to their possible risks to health and environment the microorganisms have been classified in to different risk groups.

BACTERIA

Risk group 1

Contains organisms 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
Bradyrhizobium 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 8043
Escherichia coli ATCC 9637, CCM28, NCIB 8743, B, K12 and derivatives
Erwinia spp. Except chrysanthemi, amylovora and herbicola
Gluconobacter
Klebsiella planticola

Lactobacillus
acidophilus/bavaricus/brevis/bucneri/casei/cellobiosis/fermentum/helveticum/sake
Lactococcos 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

Risk group 2

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 (f.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 corrodens
Enterobacter spp.
Enterococcus faecalis
Eperythrozoon spp.
Erysipelothrix rhusiopathiae/tonsillarum
Escherichia coli (enteroinvasive, enteropathogenic, enterotoxic etc. strains)
Flavobacterium meningosepticum
Haemophilus spp.
Klebsiella, Pathogenic spp,
Legionella spp.
Leptospira interrogans
Listeria monocytogenes
Moraxella spp.
Neisseria gonorrhoeae/meningitidis
Nocardia brasiliensis/asteroides/farcinica/nova
Pantoea agglomerans
Pasteurella spp.
Peptococcus spp.
Peptostreptococcus spp.
Propionibacteria spp.
Proteus spp.
Providencia spp.
Pseudomonas aeruginosa/pseucomallei
Rhodoccoccus 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*

Risk group 3

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
Chlamydia psittaci
Coxiella burnetii
Franciscela tularensis
Mycobacterium africanum/tuberculosis
Mycobacterium avium/chelonae/fortuitum/marinum/scrofuiaceum/ulcerans
Mycoplasma spp.
Pseudomonas mallei
Rickettsia
akari/australis/bellii/canada/conori/montana/parkeri/sibirica/tsutsugamushi/typhi
Treponema pallidum/pertenue
Yersinia pestis

FUNGI

Risk group 1

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
Rhodotorula glutinis
Saccharomyces cerevisiae
Schizosaccharomyces 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

Risk group 2

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
concentricum/erinacei/equinum/gourvillii/megninii/mentagrophytes/rubrum/
schoenleinii/smii/soudanense/tonsurans/verrucosum/violaceum/yaoundei
Xylophora carrionii

Risk group 3

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

VIRUSES

Risk group 1

-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

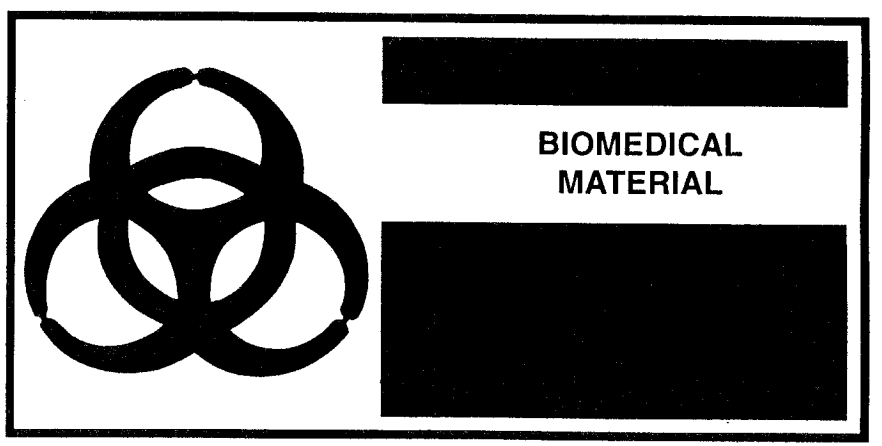
Risk group 2

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
Kilham rat virus
Cowpox virus
Lumpy skin disease virus
Minute virus in mice
Measles virus
Mumps virus
Murine pneumoniae 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

Risk group 3
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



The Universal Biohazard Sign

ANNEX 6

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 nondetrimental 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.

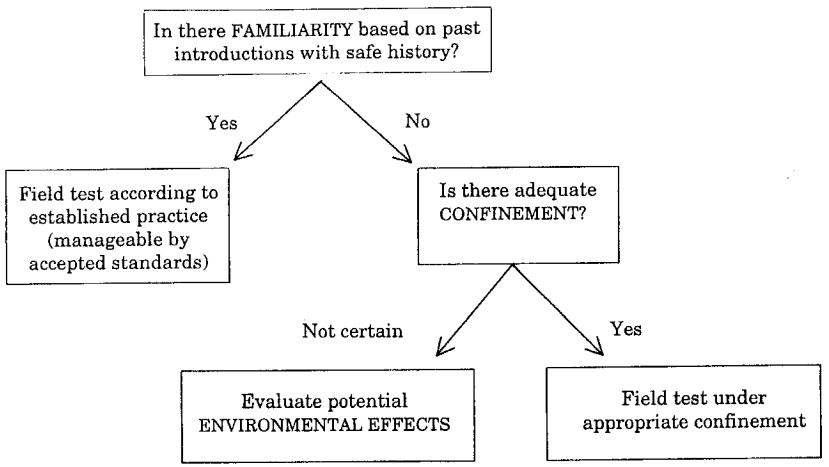


Fig. 1. Framework to assess field testing of genetically modified plants

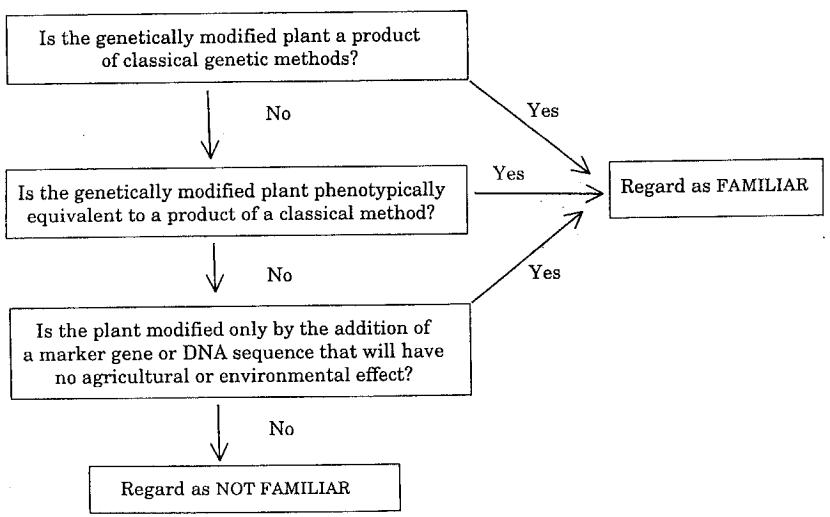


Fig. 2. Familiarity tests for genetically modified plants

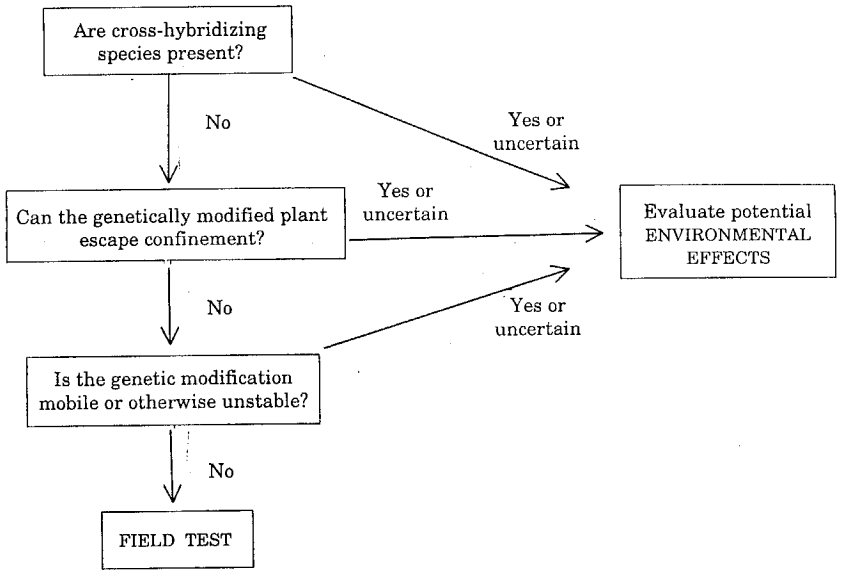


Fig. 3. Confinement tests for genetically modified plants

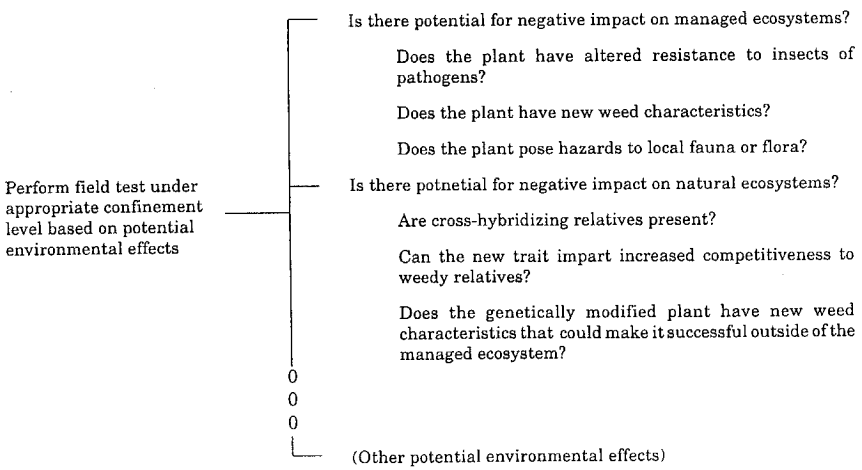


Fig. 4. Potential environmental effects : appropriate questions for specific applications to be added by users of the framework for release of genetically modified plants.

As data based on field tests accumulate, it may be desirable to lessen the confinement requirements so that a plant can be used in a crop improvement program. Field-test results need to be assesed 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.

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

Framework to assess field testing 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 modifications in microbial strains that can be characterized more fully, in some cases to the level of the DNA sequence. This reduces the degree of uncertainty associated with any intended application. The new methods have greater power because they enable scientists to isolate genes and transfer them across natural barriers.

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

The next step after laboratory experimentation is to test modified microorganisms in the field, and establishing a scientifically based framework for decisions on field testing has been a primary purpose in this report. No adverse effects of introductions have been seen and an extensive body of information documents safe introductions of some microorganisms, such as the rhizobia, mycorrhizal fungi, baculoviruses, *Bacillus thuringiensis*, and *Agrobacterium*.

However, less is known about field tests of microorganisms than of plants. Thus, for unfamiliar applications, it is prudent to prepare for the control of the introduced microorganisms.

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

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

The framework neither distinguishes between classical and molecular methods of genetic manipulation, nor between modified and unmodified genotypes. The framework is product rather than process oriented, focusing on the properties of the microorganisms 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 possible, simple and readily identifiable criteria for oversight is recognized.

Terms such as "uncertainty", "sufficient" and "significant" are used in the framework without precisely defining their quantitative limits. Any specific numerical values assigned would be arbitrary and subject to disagreement, as some underlying variables may be difficult to quantify precisely. In the final

analysis, assignment of risk categories must include a rational examination of the relevant scientific knowledge for each introduction.

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

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 famillar 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 microoorganisms, and hence its potential effects.

Specific criteria for evaluating control of the microorganism after it is introduced must include the potentials for persistence of the introduced microorganisms, genetic exchange between the introduced and indigenous microorganisms, and spread of the introduced microorganisms to non-target environments (Fig. 7). A series of questions to be addressed in evaluating the potential for unwanted persistence of an introduced microorganism is illustrated in Fig. 8.

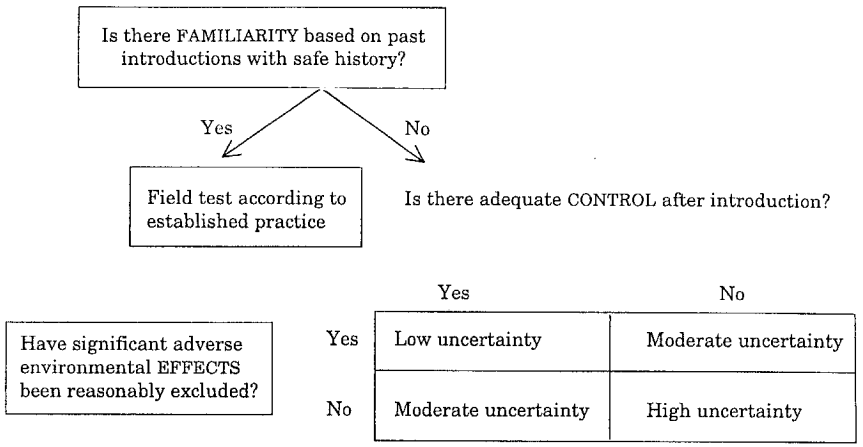


Fig. 5. Framework to assess field testing of genetically modified microorganisms

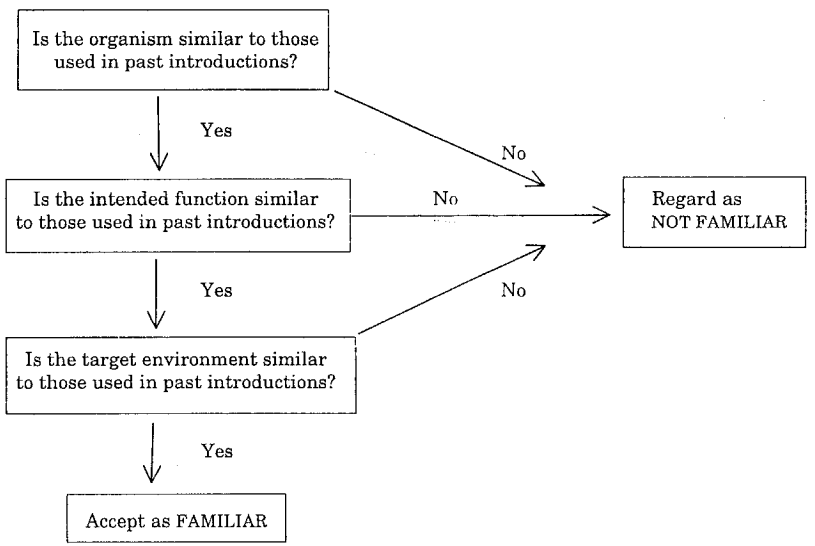


Fig. 6. Familiarity tests for genetically modified microorganisms

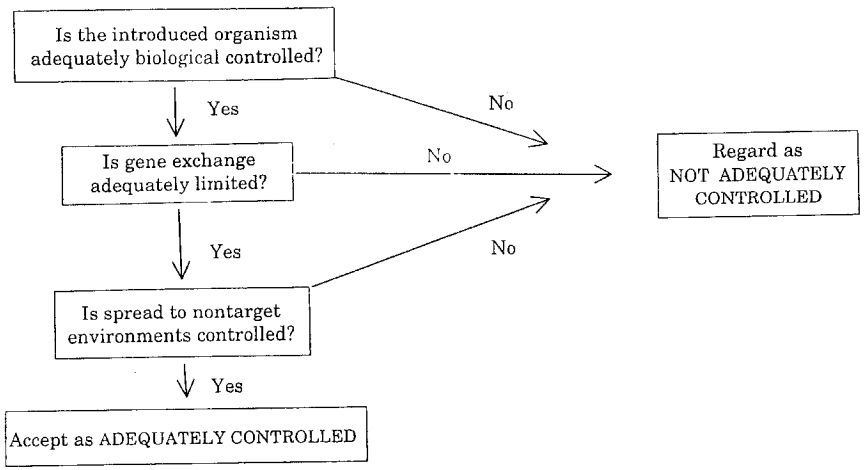


Fig. 7. Control : Appropriate questions for specific applications to be added by users of the framework for the release of genetically modified microorganisms.

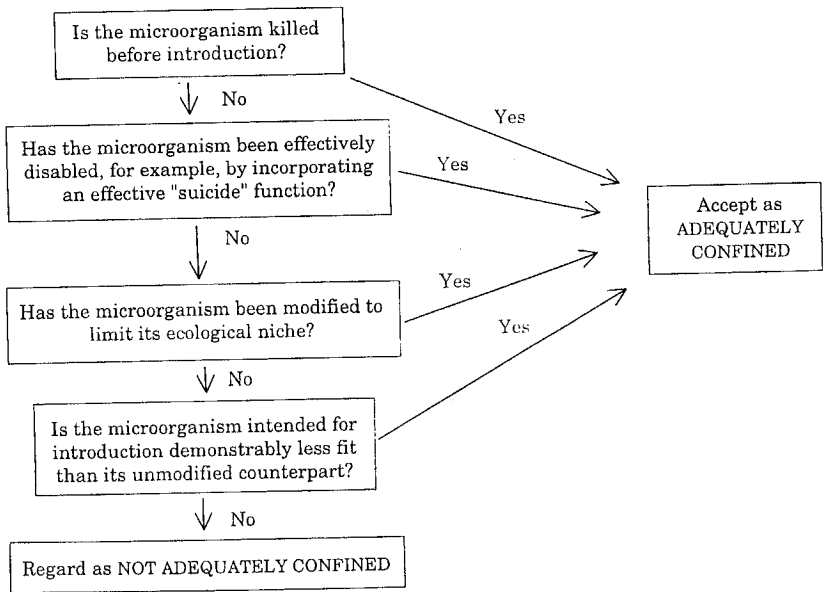


Fig. 8. Biological confinement : Appropriate questions for specific applications to be added by users of the framework for the release of genetically modified microorganisms.

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.

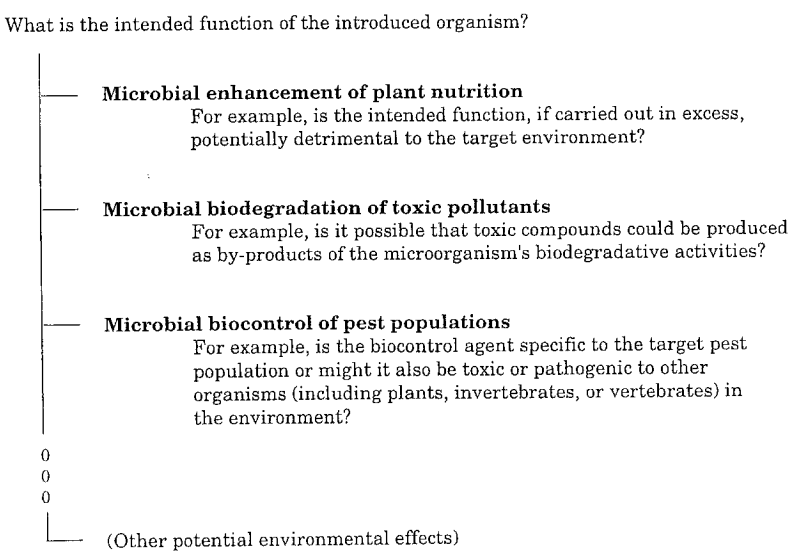


Fig. 9. Potential environmental effects : Appropriate questions for specific applications to be added by users of the framework for the release of genetically modified microorganisms.

GLOSSARY

Definition of Terms

These terms used in the Guidelines shall have the following definitions :

Amino Acid - any one of a group of 20 chemicals that are linked together in various combinations to form proteins. Each protein is made of a specific sequence of these chemicals. This unique sequence is coded for by a gene.

Anticodon - a particular combination of three bases in transfer RNA 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.

Auto-Ecology - Ecological context of both local and imported strains.

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.

Base - the units of a nucleic acid. In DNA, the four bases are adenine (A), guanine (G), cytosine (C), and thymine (T). In RNA, the base uracil (U) replaces thymine. Bases are sometimes called nucleotides.

Base-pairing rule - two bases, one in each strand of a double-sranded DNA molecule, are attracted to one another on the basis of their chemical structure: G (guanine) always pairs with C (Cytosine), and A (adenine) pairs with T (thymine) in DNA or with U (uracil) in RNA. Based on the sequence of bases in one strand of DNA, it is possible to predict the sequence in the opposite, complementary stand.

Biohazard - potential danger posed by a living or biologically derived material.

Biosafety - the policies and procedures adopted to ensure the environmentally safe applications of biotechnology.

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.

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

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

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

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

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

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

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

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.

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.

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

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

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

GMO - "GMO" means 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 new substances or perform new functions.

GMO-Products - the products involving Genetically Modified organisms (GMO's) can be grouped into two (a) where GMO's are used in the process of production byt 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 liek resistance to insect, pests or virus etc.).

Host-vector (HV) system - a microbial strain (host) and its compatible DNA carrier(s) (vector). 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.

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

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.

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

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

Ligase - an enzyme that joins the ends of DNA molecules together. These enzymes are essential tools in genetic engineering.

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

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.

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.

Pathogen - a disease-causing organism.

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

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

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.

Phage - eating or destroying characteristic of a bacterial virus.

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. thereof.

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

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

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

Recombinant DNA (rDNA) - a DNA molecule into which a foreign DNA has been inserted.

Regulated article or material - any organism which has been altered or produced through genetic engineearing, 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.

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.

Responsible individual - someone who has control and who will maintain control over the introduction of the regulated anticle 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.

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

RFLP - 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.

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

Species - reproductive communities and populations that are distinguished by their collective manifestation of ranges of variations with respect to many diffferent characteristics and qualities.

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

Transcription - the process of converting information in DNA into information contained in messenger RNA.

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

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

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

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

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.

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

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