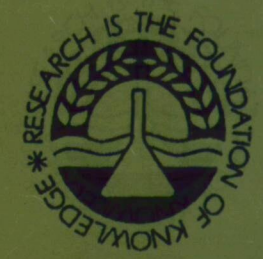
GUIDELINES

FOR THE SAFE USE OF

RECOMBINANT DNA TECHNOLOGY

IN THE LABORATORY



National Science Foundation

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National Science Foundation
Vidya Mawatha
Colombo 07

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First published 2003

ISBN 955-590-037-X

Printed and Published by
National Science Foundation
Vidya Mawatha
Colombo 07
Sri Lanka

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PREAMBLE

Research in Recombinant DNA (rDNA) is making its way into the lives of people all over the world in one way or another. Particularly, since Cohen and Boyer (1973) discovered the recombinant DNA technology, it has developed into a powerful tool that has become indispensable in modern science and biomedical research. Its greatest impact has been in the disciplines of medicine and agriculture, where it has been utilized to save lives or improve the quality of human life. Others have utilized it to unravel the mysteries of the biological universe that would not have been otherwise possible. These developments in rDNA work have also witnessed the unprecedented growth in the biomedical and related industries, propelling a new thinking era, prioritizing research and development within countries.

This powerful technology, manipulated by the human hand and mind, appears to have no bounds. In today's increasingly complex world, people have to make difficult choices in life, for which adequate information and skills are required to make that final decision (life threatening situations like disease, starvation etc.). Consequently, the impact on the society at large has also heightened, with controversial viewpoints arising between activities involving the artificial transfer and natural transfer of genetic material. Thus, research in this area of science is in the public arena, with scientists, politicians, media and the public taking an interest in its future direction.

Currently, there is a great public debate on the relative merits of this technology. There are those who are cautioning against over-simplification, uncertain knowledge and imperfect understanding of issues related to biotechnology, and others advocating its potential, for the immediate good of humankind. However, most would agree that the technology should be used prudently, as it tampers with the blueprint of life of any organism and brings about an artificial mixing of DNA of different organisms that would otherwise have been confined to naturally selected compartments, while others are happy to allow nature to operate the selective processes, as constantly governed by the phrase "nature knows best". Therefore, scientists engaging in rDNA work have come under

close scrutiny, and on their part have attempted to incorporate the necessary ethical and legal factors as checks and balances to minimize risks in research related to biotechnology.

The rDNA technology involves three essential components. A selected DNA sequence of the donor (a DNA sequence naturally occurring in an organism or synthetic sequence), a vector that is usually a virus and a plasmid or an artificial carrier that aids in the introduction of the donor sequence into the recipient host, which could range from a microbial cell to plant or animal host. In all of these components, the DNA molecule is targeted for manipulation and as such, safety considerations must encompass hazards posed by all of the three components. The National Science Foundation of Sri Lanka (NSF) recognizes in principle that such activities involve potential risks biologically, ecologically and socially; therefore, the NSF advocates good safety practices, and adherence to internationally accepted guideline for safe use of rDNA material. It also accepts that it has a responsibility to foster good laboratory practices amongst scientists in order to minimize dangers arising as a result of such work.

As a first step towards this national issue, the NSF has undertaken the task of outlining safety guidelines, relevant to Sri Lanka, based on the internationally accepted guidelines. This does not in any way attempt to hinder the progress of work of scientists, but is meant to help them address the potential hazards and negative issues of rDNA work, so that they would be thought of prior to the development, testing and use of rDNA molecules with a view to minimizing dangers.

The NSF identifies two levels of rDNA activities at which safety guidelines will be operative; those that are being conducted at the laboratory level and those that are being released to the environment. The task for formulating guidelines for safe use of rDNA molecules in the laboratory was assigned to the Working Committee on Biotechnology at the NSF. Prior to formulating such guidelines, the NSF conducted a national survey in the year 2001 to assess the current status of rDNA related activities in Sri Lanka. A total of 95 scientists representing 28 Departments from 09 Universities, 10 Research

Institutions and 03 Government Departments, responded to the questionnaire of the survey. The number of laboratory personnel engaged in rDNA related work was estimated to be 321 which includes the above mentioned 95 scientists. Of the Research & Development categories identified by the NSF for Sri Lanka (Genetic engineering in agriculture/DNA markers for breeding; DNA/immunodiagnostics; vaccines; gene therapy; environment biotechnology; industrial/pharmaceutical biotechnology; food biotechnology), DNA/Immunodiagnostics ranked the highest with 63% involvement. Only 18% of the scientists were engaged in genetic engineering related to agriculture/DNA markers for breeding. Gene therapy is currently not being done in Sri Lanka and the rest of the categories scored for less than 5% involvement by scientists. In terms of specific activities, extraction of DNA/RNA/protein and PCR assays/DNA sequencing were being carried out by 80 % of the scientists. More than 50% of the investigators were involved in culturing of microorganisms and handling of pathogens/vectors/host systems. Each of the activities identified as, use of EMO/GMO's and cloning vector/host systems, production of recombinant molecules, radiolabelling biomolecules and ELISA assays were being carried out by 40% of researchers. Scientists engaging in the production of monoclonal antibodies, and transgenic animals/plants and use of fermenters were few (10%). From this survey it can be concluded that the current status of the rDNA of work in Sri Lanka can be regarded as being in its infancy compared to the West and the Region. Most activities are confined to the laboratory level of research, with negligible amounts being carried out at field or large-scale production level.

In formulating the rDNA safety guidelines for Sri Lanka, the NSF took into consideration the guidelines currently in use in other countries. In particular, the guidelines outlined by the Health and Safety Commission's Advisory Committee on Genetic Modification, UK; Department of Biotechnology, India and the National Institute of Health, USA, were noted and modified as necessary to be applicable to Sri Lanka. The Working Committee on Biotechnology of the NSF prepared the 1st Draft of the rDNA safety guidelines in November 2001, the 2nd draft in December 2001 and eventually the 8th Draft was submitted in May 2002. This was circulated among scientists involved in rDNA work for

any suggestions for improvement. General acceptance of the guidelines by the research community was essential for its successful implementation. Therefore, the NSF organized a workshop to adopt the guidelines in February 2003 with the participation of scientists who had contributed their comments to improve the contents of the guidelines.

In order to establish safe laboratory procedures by the investigators, the NSF urges the institutions to set-up biosafety committees that would be responsible for compliance with these guidelines.

The NSF duly recognizes the untiring efforts of Drs (Mrs) Maya B. Gunasekara and Priyani Amerasinghe and the inputs of other members of the Biotechnology Committee in formulating the guidelines and completing the document in a very short period.

CHAPTER 1

INTRODUCTION

1.1.0.0 DEFINITION OF RECOMBINANT DNA MOLECULES

In the context of the guidelines stated herein, recombinant Deoxyribo Nucleic Acid (rDNA) molecules are defined as **either,**

(i) Molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate either autonomously in a living cell or as an integral part of the genome of host,

or

(ii) Molecules that result from the replication of those described in (i) above.

The definition will also include all types of cell fusion (micro-injection of DNA, RNA, parts of chromosomes or whole chromosomes); genetic engineering including self cloning, deletion & cell hybridization; transformation and other types of virus or pathogen introduction into unnatural hosts.

1.2.0.0 GENERAL APPLICABILITY OF GUIDELINES

The guidelines are applicable to all laboratory research and other laboratory activities involving rDNA molecules in Sri Lanka.

1.3.0.0 SAFETY CONSIDERATIONS

The guidelines are for the safe use of rDNA technology in the laboratory. The safety considerations herein are presented under three main areas of research:

(A) Genetic manipulation of microorganisms (Chapter 2) including animal & plant viruses and viral vectors (Chapter 3)
(B) Genetic manipulation of plants (Chapter 4)
(C) Genetic manipulation of animals (Chapter 4)

For the convenience of the "investigator"(s) undertaking rDNA work, the procedure to be followed before commencing any rDNA work, is briefly outlined below.

(i) A risk assessment of the proposed rDNA work needs to be carried out by the "investigator". An "initial risk assessment" of the rDNA activity, considering the type of etiologic agent or hazard, is first made as described (2.1.0.0) in the guidelines. This is followed by a comprehensive risk assessment (2.2.0.0) based on the agent itself, how it is to be manipulated and its potential effect on the environment.

Research areas (B) and (C) will embrace guidelines given for (A) in Chapters 2 & 3 together with additional safety considerations given in Chapter 4 .

(ii) Following the risk assessment, the "investigator" has to assign the appropriate Biosafety Containment Level (BCL) required for the proposed rDNA work.

Four Biosafety Containment Levels (BCL 1-4) are described in the guidelines (2.3.3.0) for activities involving Genetically Modified Microorganisms (GMMs) including viruses. Details of containment measures that should be adopted for each BCL are given in Chapter 6. Additional containment measures that will be required for activities involving Genetically Modified (GM) plants and animals are described separately in Chapter 4 (4.1.2.0 and 4.2.2.0).

(iii) The assignment of the BCL determines the notification requirements of the proposed rDNA work of the "investigator", to the relevant advisory body. Briefly, for the purpose of notification, guidelines stipulate three categories of rDNA activities (2.4.0.0) as follows:

rDNA Category I - Experiments that are considered under BCL 1 are exempt from notification to the advisory body.

rDNA Category II - Experiments that are considered under BCL 2, 3 & 4, except those activities listed under category III, require notification to the advisory body upon commencement of work.

rDNA Category III - Includes activities that need prior approval from the advisory body before commencement. A list of experiments in this category is provided (2.4.1.3) in the guidelines. All rDNA activities involving whole plants and animals require prior approval.

(iv) Accordingly, as prescribed in the guidelines herein, the "investigator"(s) undertaking the rDNA work and the institution (s) where the work is to be performed must establish procedures for the safe conduct of all rDNA research activities.

1.4.0.0 IMPLEMENTATION

An institutional mechanism of framework for implementation of guidelines is described (Chapter 5). The guidelines prescribe specific action needed to establish safe procedures for rDNA research. The "investigator"(s) and the institution(s) would be responsible for compliance with these guidelines and the safe conduct of work, to ensure protection of health and the environment. An Institutional Biosafety Committee (IBSC) would serve as the advisory body to all

rDNA work conducted within an institute. All institutions conducting rDNA work should establish IBSCs. At national level, a rDNA Advisory Committee (RAC) would serve as the focal point of rDNA activities in the country and provide advice and guidance to all institutions and their IBSCs and investigators. The "investigator's" role and responsibilities, the structure and composition of advisory and implementation bodies, their scope, responsibilities and functions are proposed herein for guidance.

CHAPTER 2

SAFETY CONSIDERATIONS FOR THE GENETIC MANIPULATION OF MICROORGANISMS

2.1.0.0 Initial Risk Assessment

The "investigator" may use Appendix I as a guide to make an initial assessment of risk to health and environment, by the proposed work. Appendix I consists of a list of etiologic agents, classified into four Risk Groups [RG 1 (lower risk) - RG 4 (higher risk)], based on the main criteria given below.

Criteria	Risk Group 1	Risk Group 2	Risk Group 3	Risk Group 4
Pathogenicity & capability of causing disease in humans/ animals/plants	Non –pathogenic	Pathogenic, rarely serious disease	Pathogenic, serious or lethal disease	Pathogenic, serious or lethal disease
Availability of preventive or therapeutic interventions	Not applicable	Often available	May be available	Not available

The following additional factors, based on the conditions prevalent in the country, have also been considered for the classification of an agent in a particular Risk Group.

- Prevalence of disease
- Mode of transmission
- Host range
- Availability of vector
- Epidemic causing strains

The levels of immunity, density and movement of populations and standards of environmental hygiene are additional factors that could influence this classification.

This classification does not account for instances in which an individual may have increased susceptibility to such agents e.g., pre-existing diseases, pregnancy, lactation, compromised immunity, medication, etc.

2.2.0.0 Comprehensive Risk Assessment

The "investigator" is then required to make a comprehensive risk assessment of the proposed activity. A thorough consideration of the agent itself and how it is to be manipulated and its potential effect on the environment are assessed at this stage.

For this purpose, the sequence of steps to be followed is given below.

(i) Hazard identification and assessment of degree of risk
(ii) Assignment of a provisional containment level
(iii) Consideration of environment and other activities
(iv) Assignment of the final containment level

2.2.1.0 Hazard Identification and Assessment of Degree of Risk

The following criteria need to be considered for this purpose:

(i) **Hazards associated with the host/recipient**: Pathogenicity, virulence, infectivity, toxin production and allergenicity.

(ii) **Hazards arising directly from DNA insert**: This is primarily concerned where the product of the inserted gene has biological properties which may give rise to harm such as toxins, cytokines, allergens, hormones, etc. In the event of the insert not being expressed or the expressed product being in an inactive form, it is unlikely to cause harm.

(iii) **Hazards arising from the modification** : Any alterations to existing pathogenic traits (eg: increase in infectivity or pathogenicity of organism).

In the case of microorganisms other than viruses infecting eukaryotes, the overall degree of risk is assessed by considering three factors, namely, access factor, expression factor and damage factor. These three factors are not applicable for viruses infecting eukaryotes. Additional guidelines for the risk assessment of such viruses are given separately in Chapter 3.

a. Access Factor[1]*:* is an indication of the likelihood that a Genetically Modified Microorganism (GMM) or the DNA contained within it will be able to enter the human body and

[1] In the event more than one host is used, the organism with a higher risk should be considered for the assessment.

survive there. Various routes of entry and properties of the vector e.g. mobilization functions, should also be taken into account. The access Factor for a particular vector/host system can be assessed using Table 1 of Appendix II which gives the relative values for access factors of vector and host combinations. For convenience, the vectors are categorized into three types: non-mobilizable, mobilization defective and self mobilizable. The hosts are also grouped into three: especially disabled, disabled or non-colonising, and pathogenic, colonising or wild type. Using Appendix III, which consists of a list of commonly used specific hosts and vectors in each of these categories, the "investigator" can findout to which category a specific vector and a host belongs to, for the assessment of the access factor.

b. ***Expression Factor:*** is a measure of the anticipated or known level of expression of the inserted DNA. Some relative values for the expression factor for an initial cloning experiment are given in Table 2 of Appendix II, as an example for guidance.

c. ***Damage Factor:*** is a measure of the likelihood of harm being caused to a person by exposure to a Genetically Modified Organism (GMO) of biologically active substances. This should be considered independently of access and expression factors. The recommended values for damage factors are given in Table 3 of Appendix II.

d. ***Overall Degree of Rrisk:*** is calculated by multiplying the values for access, expression and damage factors.

2.2.2.0 Assignment of Provisional Containment Level

Once a value for overall degree of risk is obtained, a provisional containment level must be assigned using the information given in Table 4 of Appendix II. See 2.3.0.0 and Chapter 6 for details of containment.

2.2.3.0 Assignment of the Final Containment Level

A final assessment of risk is made by comparing the provisional containment level (2.2.2.0) and the risk to the environment (2.2.3.1).

2.2.3.1 Consideration of Environment and Other Activities

The provisional containment level has to be further adjusted, if necessary, after considering the environment and other activities. At this point, it is necessary to double-check and ensure that all hazards are properly controlled by the proposed containment. In order to achieve this, the likelihood of hazard and the consequence of hazard need to be evaluated. A few worked-out examples for the estimation of likelihood and consequence of hazard are given in Table 5, Appendix II.

(i) Assessment of Likelihood of Hazard

The "likelihood" is the probability and frequency of hazard(s) being manifested. The likelihood is expressed as "high", "medium", "low" or "negligible". The following are the key factors considered for the assessment:

a. ***The nature of the work*** (e.g. scale, etc.,).

b. ***Non-routine/other procedures***, which might significantly affect risk associated with the GMO (e.g. procedures that give rise to aerosols, etc.,).

c. ***Characteristics of the environment likely to be exposed to the GMO***: climatic, geographical and soil conditions and the types of flora & fauna of the receiving environment [e.g. in the event the GMO is accidentally released, consider how the GMO will affect other biological systems (all microorganisms, animals and plants, with special attention to indigenous biological systems and endangered species)].

(ii) Assessment of Consequence of Hazard

After the likelihood of all hazards is assessed, the consequence of each hazard has to be estimated. The consequence will also depend to a very large extent on the receiving environment. The consequence is expressed as being "severe", "medium", "low" or "negligible".

(iii) Management of Risk

Based on the likelihood of hazard, and the consequence of each hazard, the degree of risk posed by each identified hazard can be estimated. If the consequence of a hazard is "negligible" or "low", then even if the likelihood of hazard was "high", the estimated risk of the harm is "low" (see Table 6, Appendix II for guidance). The 'investigator' should then re-evaluate whether the containment level assigned in 2.2.2.0 is adequate to protect the environment. If all risks are "low" or "negligible", then no additional control measures are necessary. If any risk exceeds these levels, then the containment level should be increased or additional control measures should be adopted so as to reduce all risks to "low". Where appropriate interactive exchange of views and opinions are recommended.

Having considered the provisional containment level and the risk to the environment, set the appropriate Biosafety Containment Level (BCL) for the experiment. Guidelines for containment are given in the next section (2.3.0.0) of this chapter. Note that the BCL required may be equivalent to the Risk Group classification of the agent (initial risk assessment) or it may be raised or lowered as a result of the comprehensive risk assessment. Careful consideration should also be given to the types of manipulation planned for some high Risk Group agents.

The importance of this final assignment of BCL is two-fold.

- It determines the notification requirements for the proposed rDNA activity (see section 2.4.0.0 of this chapter).
- It determines the containment measures that the "investigator" must adopt for the safe conduct of the proposed rDNA activity.

The guidance provided in sections 2.1.0.0 and 2.2.0.0 is expected to enable the "investigator" to complete the risk assessment of the proposed rDNA activity. Two worked-out examples of risk assessment are also given in Appendix IV for guidance.

2.3.0.0 CONTAINMENT

The term "containment" is used in describing the safe methods for managing infectious agents and genetically altered agents in the laboratory environment where they are being handled or maintained.

2.3.1.0 Purpose of Containment

The purpose of containment is to reduce exposure of laboratory workers, others (human subjects other than laboratory workers and animals), and the outside environment, to potentially hazardous agents.

2.3.2.0 Types of Containment

2.3.2.1 Biological Containment

In this context, the vector (plasmid, organelle, or virus) for the recombinant DNA and the host (bacterial, plant, or animal cell) in which the vector is propagated in the laboratory will be considered together. Biological containment is achieved by selecting (or constructing) an appropriate vector-host system that limits the infectivity of vector to specific hosts and controls the host-vector survival in the environment. For example, a high level of containment can be achieved by using disabled host-vector systems (Appendix III).

2.3.2.2 Physical Containment:

The objective of physical containment is to confine recombinant organisms, thereby preventing the exposure of the researcher and the environment to harmful agents. Physical containment is achieved through the use of good laboratory practice, containment equipment and special laboratory design/facility design.

Protection of personnel and the immediate laboratory environment from exposure to infectious agents is achieved by good microbiological practices and by the use of appropriate safety equipment (Primary containment). The protection of the environment external to the laboratory from exposure to infectious material is achieved by a combination of facility design and operational practices (Secondary containment).

(i) Good Laboratory Practices

- Strict adherence to good microbiological practices and techniques
- Awareness of potential hazards
- Providing appropriate training for personnel involved in rDNA work
- Selection of safety practices in addition to the standard laboratory practices if required
- Developing/adopting a biosafety or operations manual which identifies the hazards
- Appointment of a safety officer or person to monitor activities within the laboratory.

(ii) Containment Equipment (Primary Containment)

Containment equipment includes biological safety cabinets (BSC) and a variety of enclosed containers (e.g. safety centrifuge cups). The biological safety cabinet is the principal device used to provide containment of infectious aerosols generated by many microbiological procedures. Three types of BSCs (Class I, II and III) are used in microbiological laboratories. Safety equipment also includes items for personal protection such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, etc.

(iii) Facility Design (Secondary Containment)

The design of the facility is important in providing a barrier to protect persons working in and outside the facility or the laboratory from infectious agents which may be accidentally released from the laboratory. There are three types of facility designs: viz. the Basic Laboratory (for micro-organisms categorized under RG 1 and 2), the Containment Laboratory (for RG 3) and the maximum Containment Laboratory (for RG 4).

2.3.3.0 Biosafety Containment Levels (BCLs)

Biosafety containment levels describe laboratory practices, containment equipment and facilities appropriate for each risk group of micro-organisms, as per recommendations of the World Health Organization (WHO). Accordingly, the BCLs are also sometimes designated as P1<P2<P3<P4 corresponding to the risk groups 1-4, where 'P' stands for disease-producing potential. However, the proposed containment levels

for rDNA work also take into consideration the source of the donor DNA and the risk group that it belongs to, and the effect of any genetic manipulation. Hence, the BCL may be equivalent to the risk group classification of the agent or the BCL may be raised or lowered after the comprehensive risk assessment of the rDNA activity.

Containment measures that could be used at the four BCL are given in Appendix V1 and in Chapter 6.

2.3.3.1 BCL 1

The practices, safety equipment and facilities in this level are appropriate for undergraduate & secondary educational training laboratories and other facilities which use defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans.
No special accommodation or equipment is required but the laboratory personnel are required to have specific training and to be supervised by a scientist with general training in microbiology or a related science.

2.3.3.2 BCL 2

The practices, safety equipment and facilities in this level are applicable in clinical, diagnostic, teaching and other facilities in which work is done with a broad spectrum of indigenous moderate-risk agents present in the community and associated with human disease of varying severity.

Laboratory workers are required to have specific training in handling pathogenic agents and to be supervised by competent scientists. Accommodation and facilities including safety cabinets are prescribed, especially for handling large volumes and high concentrations of agents where aerosols are likely to be created. Access to the laboratory is controlled. Generally, safety cabinets of category II are used. It is assumed that those engaging in activities under BCL 2 are familiar with the requirements of BCL 1.

2.3.3.3 BCL 3

The practices, safety equipment and facilities in this level are applicable to clinical, diagnostics, teaching research or production facilities in which work is done with indigenous or exotic agents where the potential for infection by aerosols is real and the disease may have serious or lethal consequences.

Personnel are required to have specific training in work with these agents and to be supervised by scientists experienced in this area of microbiology. Specially designed laboratories and precautions including

the use of safety cabinets (Category III or above) are prescribed and the access is strictly controlled. It is assumed that those engaging in activities under BCL 3 are familiar with the requirements of BCL 1 and BCL 2.

2.3.3.4 BCL 4

The practices, safety equipment and facilities in this level are applicable for work with dangerous and exotic agents, which pose a high individual risk of life-threatening disease.

Strict training and supervision are required and the work is done in specially designed laboratories under stringent safety conditions, including the use of safety cabinets and positive pressure personnel suits. Access is strictly limited. A specially designed suit area may be provided in the facility. Personnel who enter this area wear a one-piece positive pressure suit that is ventilated by a life support system. A life support system is required with alarms and emergency break-up breathing air tanks. Entry to this area should be through an airlock, fitted with airtight doors. A chemical shower should be provided to decontaminate the surface of the suit before the worker leaves the area. The exhaust air from the suit area requires to be filtered by two sets of HEPA filters installed in a series. A duplicate filtration unit, exhaust fan and an automatically starting emergency power source should be provided. The air pressure within the suit area should be lower than that of any adjacent area. Emergency lighting and communication systems should be provided. All penetrations into the internal shell of the suit area should be sealed. A double-doored autoclave must be provided for decontamination of disposable waste materials from the suit area. It is assumed that those engaging in activities under BCL 4 are familiar with the requirements of BCL 1, BCL 2 & BCL 3.

2.4.0.0 NOTIFICATION OF rDNA RESEARCH ACTIVITIES

Once the BCL required for the proposed activity is determined, the "investigator" is required to give written notification (Appendix VI) of the proposed rDNA activity to the relevant authority, the Institutional Biosafety Committee (IBSC) [1], if necessary, as stipulated by the guidelines given below. The different stages of experimentation (i.e. large scale experimentation, release to the environment and importation and shipment) may also require notification (see under specific sections in this Chapter). The "investigator" (s) and the institution(s) where the work is to

[1]Institutional Bio-Safety Committee (IBSC) is to be constituted in all centers engaged in rDNA laboratory activities. It is the responsibility of the Head of the institution to setup an Institutional Biosafety Committee (see Chapter 4 for guidance).

be performed, must also take appropriate action to adopt procedures given in the guidelines for the safe conduct of all rDNA activities.

The guidelines stipulate three categories of rDNA activities for the purpose of notification.

2.4.1.1 Category I

Experiments that are considered under BCL 1 are exempt from notification of IBSC[1]
These experiments include:

(i) Self-cloning experiments using strains, and also inter-species cloning belonging to organisms in the same group, as listed in Appendix I.

(ii) Organelle DNA, including those from chloroplasts and mitochondria.

(iii) Host-vector systems consisting of cells in culture and vectors, either non-viral or viral, containing defective viral genomes (except from cells known to harbour RG 3, RG 4 and special category etiologic agents listed under Appendix I.

2.4.1.2 Category II

Experiments that are considered under BCL 2, 3 & 4 except those activities listed under category III, require notification of IBSC.
These include:

(i) Experiments falling under BCL 2,3 & 4.

(ii) Experiments wherein DNA or RNA molecules derived from any source except from viral genomes in eukaryotes may be transferred to any non-human vertebrate or any invertebrate organism and propagated under conditions of physical containment appropriate to the organism under study.

(iii) Experiments involving non-pathogen DNA vector systems and regeneration from single cells.

[1]Institutional Bio-Safety Committee (IBSC) is to be constituted in all centers engaged in rDNA laboratory activities. It is the responsibility of the Head of the institution to setup an Institutional Biosafety Committee (see Chapter 4 for guidance).

(iv) Large-scale use of recombinants made by self-cloning in systems belonging to exempt category (e.g. *E. coli, Saccharomyces*, and *B. subtilis)*

2.4.1.3 Category III

The following experiments need prior approval from the IBSC before commencement.

(i) All toxin gene cloning experiments producing LD_{50} less than 50 ug/kg of body weight of vertebrates or large scale growing (a list of toxins classified based on their potential toxicity is given in Appendix III).

(ii) Cloning of genes for vaccine production: the containment should be decided by the rDNA Advisory Committee (RAC) on a case by case basis on experiment utilising DNA from non-defective genomes of organisms recognised as pathogens. The details of the rDNA technology in the development of vaccines for human and animal health giving containment conditions for observance of safeguards in large scale operations are given in Chapter 5.

(iii) Experiments dealing with mosquito and tick DNA cloning should be prescribed on a case by case basis since these are natural vectors for certain endemic viral and parasitic diseases.

(iv) Genes coding for antibiotic resistance into pathogenic organisms which do not naturally possess such resistance.

(v) Introduction of rDNA molecules containing complete genes of potentially oncogenic viruses, into cultured human cells or transformed cellular genes.

(vi) Introduction of unidentified DNA molecules derived from cancer cells or from *in vitro* transformed cells, into animal cells. Transformation of animal cells with foreign DNA using viruses as vectors or by microinjection of DNA into eggs and pro-embryos.

(vii) Experiments involving the use of infectious animal and plant viruses in tissue culture systems.

(viii) Experiments involving gene transfer to whole plants and animals.

(ix) Cell fusion experiments of animal cells containing sequences from viral vectors if the sequence leads to transmissible infection either directly or indirectly as a result of complementation or recombination in the animals. For experiments involving recombinant DNA of higher class organisms, using whole animals will be approved on a case by case basis.

(x) All experiments involving the genetic manipulation of plant pathogens and the use of such genetically manipulated plant pathogens.

(xi) Experiments requiring field testing and release of rDNA containing microorganisms and plants as specified in the guidelines given by the "National guidelines for import and planned release of genetically modified organisms and products there of" (Draft document is in preparation by the Ministry of Environment and Natural Resources).

(xii) Diagnostics: No major risk can be foreseen on diagnostics involving *in-vitro* tests. For diagnostics involving *in-vivo* tests, specific containment levels have to be prescribed on a case by case basis.

(xiii) Experiments involving "Gene therapy".

(xiv) All rDNA experiments involving operations of more than 10-litre capacity (large scale experiments).

2.5.0.0 LARGE SCALE EXPERIMENTS

Experiments beyond 10-litre capacity for research as well as industrial purposes are included in the category of large-scale experimentation/ operations. For such activities, it is recommended that one should seek approval of the IBSC. For good large-scale practice (GLSP) as well as levels of containment, the following principles of occupational safety and hygiene should be applied.

(i) To keep workplace and environment exposure to any physical, chemical or biological agent at the lowest permitted level.

(ii) To exercise engineering control measures at source and to supplement these with appropriate personal protective clothing and equipment when necessary.

(iii) To test adequately and maintain control measures and equipment.

(iv) To test when necessary for the presence of viable organisms cultured in large scale, outside the primary physical containment.

(v) To provide training for personnel.

(vi) To formulate and implement a local code of practice for the safety of personnel.

2.5.1.0 The Safety Criteria for Large-Scale Practices

The physical containment conditions that should be ensured for large-scale experiments and production activities are given in Chapter 5. The factors to be considered are briefly outlined below:

(i) The host organism should not be a pathogen, should not contain adventitious agents, and should have an extended history of safe use, or have a built-in-environmental limitation that permits optimum growth in the bioreactor but limited survival with no adverse consequences in the environment.

(ii) The vector/insert should be well characterized and free from known harmful sequences; the DNA should be limited in size as much as possible to perform the intended function; should not increase the stability of the recombinant in the environment unless that is a requirement of the intended function; should be weakly-mobilizable or non-mobilizable and should not transfer any resistance markers to microorganisms not known to acquire them naturally if such acquisition could compromise the use of a drug to control disease agents in human or veterinary medicine or agriculture.

(iii) The genetically manipulated organism should not be a pathogen and should be assessed as being safe in the bioreactor as the host organism, and without adverse consequences to the environment (Appendices I & III may be used for guidance).

2.6.0.0 RELEASE TO THE ENVIRONMENT

Depending on the types of organisms handled and assessment of potential risks involved, appropriate containment facilities must be provided to ensure safety of workers and to prevent unwanted release to the environment.

Bio-waste resulting from laboratory experiments, and from industrial operations should be properly treated so that the pathogenicity of genetically engineered organisms are either destroyed or rendered harmless before disposal to the

environment. Special facilities should be created for disposal of experimental animals: incineration of all refuse and carcasses is desirable.

For planned release of organisms into the environment, follow the " National guidelines for import and planned release of genetically modified organisms and products thereof" (Draft document is in preparation by the Ministry of Environment and Natural Resources).

2.7.0.0 IMPORT AND SHIPMENT

The shipment of indigenous etiologic agents, diagnostic specimens and biological products is subject to recommended packaging, labeling and shipping requirements as illustrated in Figures 2.1, 2.2 and 2.3. The "investigator" is also expected to obtain any required Import/Export license from relevant authorities, where applicable. Importation of certified vector/host systems (Appendix III) for laboratory research will be permitted without notification. Importation of GMOs for commercial and other purposes are not covered by these guidelines.

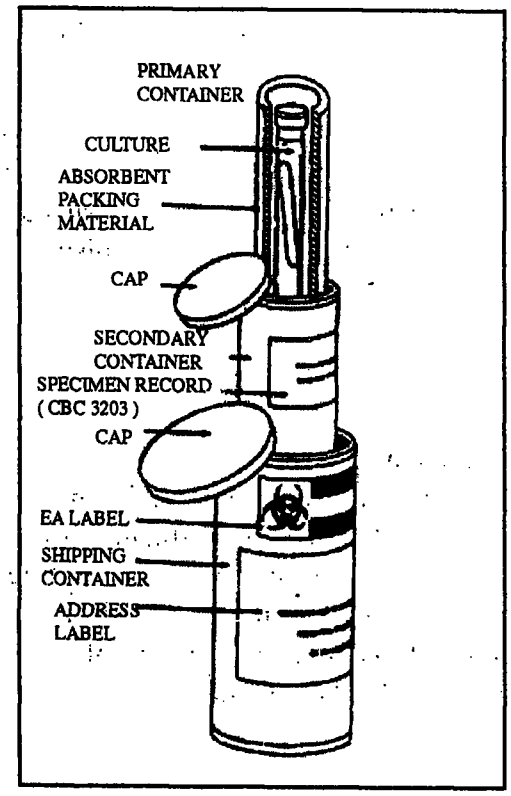

Figure 2.1: diagram illustrates packaging and labeling of etiologic agents in volumes of less than 50 ml.

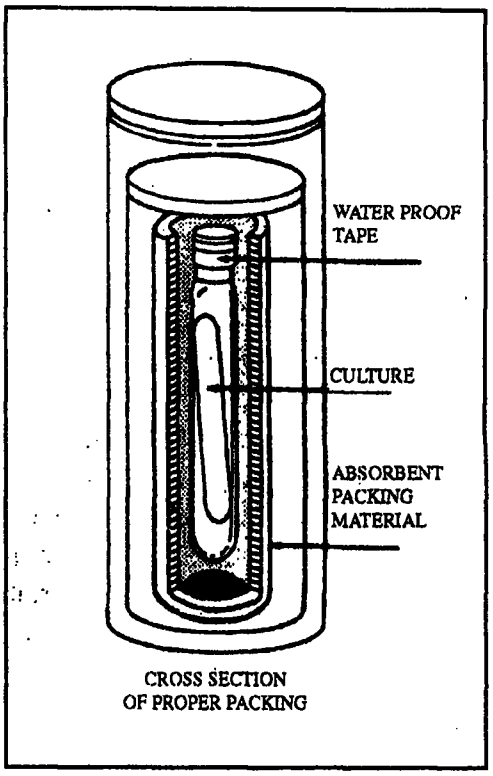

Figure 2.2: .diagram illustrates packaging and labeling of etiologic agents in volumes of less than 50 ml.

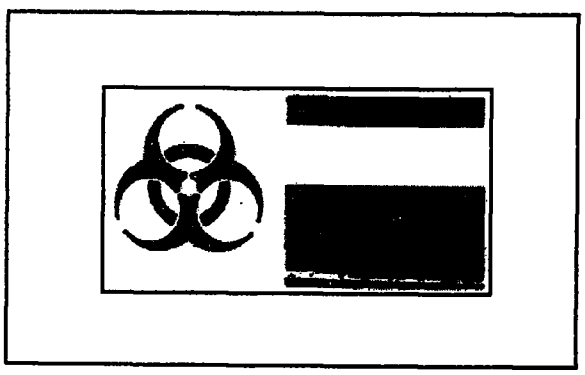

Figure 2.3: A biohazard label should be affixed to all packages containing etiologic agents

CHAPTER 3

SAFTEY CONSIDERATIONS FOR GENETIC MANIPULATION OF VIRUSES & VIRAL VECTORS OF EUKARYOTES

3.1.0.0 RISK ASSESSMENT OF GENETICALLY MODIFIED ANIMAL VIRUSES & VIRAL VECTORS

This part is intended to provide guidance on the risk assessment of work involving Genetically Modified (GM) animal viruses and viral vectors. This supplements the general guidance given in Chapter 2 for GMMs.

3.1.1.0 Additional Guidelines for Risk Assessment

For the purposes of this guidance, the term "animal" is taken in its broadest sense, and includes both vertebrates and invertebrates. The following is the recommended procedure for risk assessment:

(i) Risk assessment for human health : hazard identification, likelihood of hazard, assignment of provisional containment level, consideration of nature of activity and assignment of additional control measures

(ii) Risk assessment for environment protection

(iii) Assignment of the final containment level

3.1.1.1.1 Risk Assessment for Human Health

(i) Hazard identification

a. Hazards associated with the vector

Particular care must be given to the assessment of vectors with an actual or potential ability to infect humans or human cells. All biological agents (in this context, any virus or viral vector that may cause any infection, allergy, toxicity or any other hazards to human health) are classified into one of four hazard groups (Appendix I).

b. Viral vectors with reduced pathogenicity

By substituting, where reasonably practicable, a biological agent which is less hazardous, the risk of exposure can be reduced. For genetic modification work involving viruses with a human host range, this can be equated to a statutory requirement to, whenever possible, use disabled or attenuated viral vectors with a reduced pathogenicity. Furthermore, where appropriate, use of a vector without a human host range should be considered.

Insertion of a gene into the site of any disabling mutation is expected to reduce the likelihood that recombination events could result in the generation of replication competent virus expressing the gene, thus increasing the effective biological containment. This principle should be followed whenever practicable, especially when working with harmful genes.

Experiments using viral vectors that do not normally infect human cells in culture, and for which there is no evidence of human infection, are considered to represent a minimal risk to the operator and BCL 1 is sufficient to protect human health. A higher standard of containment may however be required to control risks to other species.

Experiments which involve DNA (or RNA) vectors derived from viruses, together with cell cultures as hosts but in which no virus particles are involved or can be produced, are covered by the guidance in Chapter 2. Note that this does not apply to the use of packaging cell lines intended to produce mature infectious virus particles.

c. Hazards arising directly from the inserted gene product

The insertion of additional nucleic acid sequences into a viral vector can give rise to potential adverse effects. These may result either from the direct effects of an expressed gene product or as a consequence of an alteration in the overall properties of the GMM (see section below). In considering the direct effects, particular attention should be paid to the level of expression and site of insertion of the gene(s) and whether there is a known or suspected pharmacological or physiological effect, including the possibility of effects other than those being sought in the construction.

Particular attention should be paid to the insertion of genes which may alter the growth, replication or differentiation of cells, for example; oncogenes, potentially oncogenic sequences, or genes encoding biologically active proteins (e.g. cytokines, growth factors or toxin) into viruses capable of infecting human cells. Work with such modified viruses may pose serious consequences for people who are occupationally infected or exposed. Additional containment and control measures over and above those required for the viral vector will generally be necessary and must be applied at the correct level following the risk assessment.

d. Hazards arising from the alteration of existing pathogenic traits

Many modifications to eukaryotic viral vectors do not involve genes whose products are inherently harmful but adverse effects may nevertheless arise as the result of exacerbation or alteration of existing pathogenic traits. This may arise as a result of the product of an inserted gene acting alongside existing pathogenic determinants. Alternatively, it is possible that modification of normal viral genes may also alter pathogenicity. In identifying any hazards associated with the modification to the virus, the following points should be considered.

- **Alteration of tissue tropism or host range**: Is there a possibility that the structure of the receptor binding site will be altered or will the product of the inserted gene be incorporated on the virus surface with the possibility of forming a novel receptor binding capacity? Cell or tissue tropism may also be affected by alterations in the transcriptional control of viral genes.

- **Increase in infectivity or pathogenicity**: Could the modified virus show an altered susceptibility to host defence mechanisms? Is the recombinant likely to have enhanced effects upon an immuno-compromised host, beyond those normally expected with the parent virus?

- **Recombination or complementation**: Could any disabling feature or attenuation of the viral vector be overcome by recombination or complementation either following accidental infection of a laboratory worker or accidental cross-contamination of cultures in the laboratory?

- **Availability of prophylaxis or therapy:** Will viral susceptibility to anti-viral drugs (where these are available) be affected by genetic modification? Can vaccination or normal immune status be expected to protect against the modified virus?

e. Transfer of harmful sequences to related viruses

Whilst the phenotype of the recombinant virus that is under construction is the primary consideration, some thought must also be given to the possibility that harmful sequences may be transferred as a result of recombination.

(ii) Likelihood of Risk

The initial stages in the risk assessment process that have been outlined above involve identifying those features of the GMM which have the potential to cause harm to humans. It is, however, recognised that in some cases, while it may be possible to draw up theoretical scenarios to suggest that a modified virus may be hazardous to human health, there can sometimes be justification to say that the likelihood of these scenarios being realised is extremely small.

Factors which come into play when considering likelihood include the analysis of the probability that rare events may occur (e.g. mutation which overcome disabling mutations) and a judgement as to the fitness (*in vivo* spread) of the modified virus.

Issues relating to the likelihood of harm arising will, by their very nature, be very difficult to handle in situations where there is no firm data on which to make a judgement. Therefore, a great deal of caution must be applied when seeking to discount, on the basis of likelihood, those predicted properties of the modified virus which have been identified as being potentially harmful. In general, the weighting given to information used in the consideration of likelihood should reflect the quality of the supporting data. Where the information is either anecdotal or based on a series of roughly-drawn assumptions, it may be necessary to assume the worst and act accordingly.

Consideration of the fitness of a virus is a legitimate part of risk assessment, but should not be based merely on supposition, but on established scientific knowledge. Until it can be demonstrated that a particular type of modification will render a virus less fit than the parental virus (for example by experimental data, or through the literature etc.) the precautionary principle should be followed. This is particularly so where counter-arguments can be made for the foreign insert giving the virus a competitive advantage.

(iii) Assignment of a Provisional Containment Level

The starting point for assignment of a provisional containment level is likely to be the biological agents hazard group (Appendix I). "Investigators" would then consider whether the genetic modifications will alter the level or nature of any hazards. In some cases, where it is predicted that the modified virus will be considerably more hazardous than the parental virus (e.g. where a harmful gene has been inserted into a replication-competent virus), it may be appropriate to assign it to a higher hazard group /

containment level than the parent. The biological agent's hazard group directly indicates the minimum containment level necessary to work with the organism and, in turn, this will give the provisional containment level.

(iii) Consideration of the Nature of the Work & the Assignment of Additional Control Measures

At this stage the control measures are checked again to ensure that all risks to humans from the actual activity are low or negligible. Aspects considered are:

a. Whether the provisional containment level identified is sufficient to control all of the potential harmful properties of the GMMs, or whether some additional measures might be required. (It is also possible that the assessment will show that some of the measures from the initial level identified are not necessary);

b. The nature of the activity to be undertaken, especially consideration of any non-standard operations.
Examples of such activities could include the following:

- Inoculation of animals with modified virus;
- The use of equipment likely to generate aerosols e.g. sonication or mixing;
- The use of high titre virus.

If it is decided that any such non-standard operations are likely to generate risks that are not accounted for in the provisional containment assigned, additional control measures should be applied. It should be decided whether the minimum requirements for the provisional containment level are adequate or whether some additional measures over and above the minimum need to be applied. It may be possible to identify some particular aspect of the experimental design or work procedures which can be improved in order to minimise the risk to human health and safety. For example, some projects may be assigned to BCL 2 with one or two additional measures taken from the requirements of BCL 3. Management systems may also need to be implemented or improved e.g. increased monitoring by internal inspections and ensuring workers are adequately trained and fully aware of the potential hazards.

3.1.1.2 Risk Assessment for Protection of the Environment

The primary consideration here is whether the virus is capable of infecting animals (vertebrates and invertebrates). If the virus cannot infect any species other than humans the risk assessment should include a statement to this effect together with some justification. For such cases it can be assumed that the risks to the environment will be negligible. If it may infect any animals (vertebrates or invertebrates), then the assessment should consider the risks posed to the environment. Attention should also be paid to any viruses which are known to be pathogenic to wildlife (vertebrates and invertebrates) and, in particular, any endangered species which could be affected. Any additional risks to the environment caused by the modification or the inserted sequence should be assessed by consideration of the following points:

(i) **Survivability:** is there a reason to suspect that the modification carried out to the virus may result in altered survivability in the environment? Special attention should be given to effects on UV tolerance, temperature and resistance to desiccation. If the virus is capable of long term survival in the environment and there are indigenous species with which it can recombine/re-assort, then further considerations will be the likelihood of harmful sequences being transferred to closely related viruses and the possibility that the selective pressures could lead to the emergence of mutant derivatives that are more harmful than the recombinant virus.

(ii) **Alteration of tissue tropism or host range:** is the modification likely to alter the tissue tropism or host range of the recombinant virus?

(iii) **Increase in infectivity or pathogenicity:** is the modification likely to increase the infectivity or pathogenicity of the virus vector? Is the modified virus likely to show altered susceptibility to host defense mechanisms?

(iv) **Effects on other organisms:** does the insert code for a protein(s) with known or suspected inhibitory, detrimental or have other physiologically active effects on other organisms? Consideration should be given to possible effects other than those being sought in the construction.

(v) **Environmental release:** are all potential routes of transmission or escape to the environment known? If so, will such routes allow the modified virus and/or its products access to the organisms in which effects may be manifested?

(vi) **Availability of control agents:** will virus susceptibility to control agents (where these are available) be affected by genetic modification? Can vaccination (in domestic animals) or normal immune status (in any animal) be expected to protect against the modified virus?

Any hazard identified from these considerations should be re-assessed by estimating the likelihood and the consequence of hazard. There may be cases where a virus is known to have limited survivability in the environment or is known not to infect Sri Lankan hosts. In such cases, the likelihood that a hazard will be realised in the environment could be considered as "low" or "effectively zero". In considering survival, it is important to determine the likely route of the virus into the environment. In an aerosol, the probability of survival may be poor, but the virus may survive well in infected animal material. The ability of the virus to infect hosts and replicate within them are also important characteristics to take into account. The assessment of risks should be as described in Chapter 2. If the modified virus has been assigned to a low level of containment on the basis of risks to human health, and the final risk in terms of environmental safety is not considered to be "negligible", additional controls may need to be adopted and the environment risk assessment repeated. The additional measures should seek to reduce the likelihood of environmental exposure. Particular attention will need to be given to the possible routes of escape including the disposal of infected material, in order to minimize risks of accidental spread of virus beyond the laboratory. For certain viruses the possibility of airborne spread will need to be considered, through ventilation systems or insect vectors, for example.

3.1.2.0 Assignment of the Final Containment Level

As described in Chapter 2 for GMMs.

3.2.0.0 RISK ASSESSMENT OF GENETICALLY MODIFIED PLANT VIRUSES

This part is intended to provide guidance on the risk assessment of work involving GM activities using plant viruses. This supplements the general guidance in Chapter 2.

3.2.1.0 Additional Guidelines for Risk Assessment

For the purposes of this guidance, the term "plant" is taken in it's broadest sense, and includes fungi, algae, mosses and ferns as well as gymnosperms and angiosperms. The term "GM virus" is used throughout the text, to

avoid confusion with "vector", which is used to describe the carrier of a virus, such as an insect. In summary, the required steps and considerations are:

(i) Risk to environment : hazard identification; estimation of likelihood and severity of the consequence; determination of a provisional containment level; consideration of the nature of the work to be undertaken and the assignment of the controls necessary to safeguard the environment;

(ii) Risk to human health and safety and the assignment of any additional containment measures to protect human health;

(iii) Assignment of the final containment level.

3.2.1.1 Environment Risk Assessment

Consideration of the predicted properties of the genetically modified virus to determine if there are any potential mechanisms by which it could represent a hazard to the environment. Estimation of the severity of any potential harm and assignment to a provisional containment level.

(i) Hazard Identification

a. Hazards associated with the virus

Any hazard identified from these considerations should be assessed as described in Chapter 2 by estimating the likelihood that identified hazards will be realised and the consequences. The possibility of accidental release (escape) and survival of the modified virus are important in assessing the risk to the envioronment.

It is generally accepted that, where possible, it is safer to use disabled or attenuated viruses with reduced pathogenicity or infectivity. The origin and mechanism of such attenuation should be well understood and will form an important part of the risk assessment. In assessing whether a viral vector is adequately disabled the possibility of reversion or complementation should be considered and it should be confirmed that the virus is disabled after modification. The likelihood of reversion will depend on the mechanism of attenuation; deletion mutants are less likely to revert to wild-type than point mutations or conditional lethal mutants.

b. Hazards arising directly from the inserted gene product

The insertion of additional nucleic acid sequences into a plant virus can give rise to potential adverse effects. These may result either from the direct effects of an expressed gene product or as a consequence of an alteration in the overall properties of the GMM . In considering the direct effects, particular attention should be paid to the level of expression and site of insertion of the gene(s) and whether there is a known or suspected physiological effect, including the possibility of effects other than those being sought in the construction.

c. Hazards arising from the alteration of existing pathogenic traits

Many modifications to plant viruses do not involve genes whose products are inherently harmful but adverse effects may nevertheless arise as a result of exacerbation or alteration of existing pathogenic traits. This may arise as a result of the product of an inserted gene acting alongside existing pathogenic determinants. Alternatively, it is possible that modification of normal viral genes may also alter pathogenicity. In identifying any hazards associated with the modification to the virus, the following points should be considered :

- **Alteration of tissue tropism or host range**: Is the modification likely to alter the tissue tropism or host range of the recombinant virus?

- **Structure of the receptor binding site**: Whether it will be altered or will the product of the inserted gene be incorporated on the virus surface with the possibility of forming a novel receptor binding capacity? Cell or tissue tropism may also be affected by alterations in the transcriptional control of viral genes.

- **Increase in infectivity or pathogenicity**: Could the modified virus show an altered susceptibility to host defense mechanisms?

d. Survivability, hazards from infectious nucleic acid

Any additional risks to the environment caused by the modification or the inserted sequences should be assessed by consideration of the following:

- **Availability of control agents**: will virus susceptibility to control agents (where they are available) be affected by the genetic modification?

- **Survivability**: Is there reason to suspect that the modification carried out to the virus may result in altered survivability in the environment? Consideration should be given to differences in structure and resilience of all virus-derived material because this may impact upon UV or temperature tolerance and the survival of infectious agents exposed to desiccation.

(ii) Consideration of the Likelihood of Hazard ;
(iii) Assignment of a Provisional Containment Level;
(iv) Consideration of the Nature of the Work to be Undertaken; and
(v) Assignment of Additional Control Measures to Protect the Environment

For the above (i)-(v), follow the guidelines described for animal viruses in section 3.1.0.0. If it is decided that any non-standard operations are likely to generate risks that are not accounted for in the provisional containment level assigned, additional control measures should be applied. Non standard operations include large scale growth of modified viruses in plants for commercial exploitation; downstream activities to harvest large scale virus production.

3.2.1.2 Risk Assessment for Human Health and Safety

(i) Identification of any hazards to humans

The most likely hazards will be where the modification results in either production of a toxin or allergen: development of biopharming production of pharmacologically or immunologically active substances in plants infected with modified viruses.

(ii) Assignment of any additional containment measures

Where a potential hazard to humans is identified, the severity of any harmful consequence and likelihood of it occurring should be estimated.

If any of the hazards identified are not controlled by the measures for environmental protection so that the level of risk to humans is low or negligible, suitable additional control measures should be assigned.

3.2.1.3 Assignment of the final containment level

As described in Chapter 2 for GMMs.

CHAPTER 4

GENETIC MANIPULATION OF PLANTS & ANIMALS

4.1.0.0 GENETIC MANIPULATION OF PLANTS

This provides additional guidance on the risk assessment activities involving GM plants. For activities involving both the risks from the GMMs (see Chapter 2) including plant viruses (see Chapter 3) infected/associated with GM plants, the risks should be assessed together to ensure that all aspects are appropriately controlled. Follow the current guidelines given by Sri Lanka Plant Quarantine Service, Department of Agriculture, for the general procedures of export and import of plants and plant products. Plant pathogens of importance to Sri Lanka are also documented in the above guidelines. Investigators should refer to Plant Protection Act and Fauna and Flora Protection Ordinance for relevant information.

4.1.1.0 Additional Guidelines for Risk Assessment

The recommended procedure is briefly outlined below:

(i) Risk assessment for environmental protection (including hazard identification; assessment of likelihood and consequences; determination of risk and assignment of risk management measures to protect the environment);
(ii) Risk assessment for human health and safety;
(iii) Assignment of the final containment and control requirements [adjusting (i) above to take account of (ii) above].

4.1.1.1 Risk Assessment for Protection of the Environment

(i) Hazard Identification

Hazards that need to be considered are:

a. ***Effect on biodiversity*** - capacity to survive, establish, disseminate, compete with and/or displace other plants.

Survival will be the key consideration. It should be interpreted in its broadest sense to include the ability to go through a full life cycle and pass on its genes. Some GM plants may not be able to survive in the wider environment and can be considered safe. However, others will show varying degrees of ability to survive: for instance, some may take root and grow but not flower or produce seed. For any GM plant

which can survive, even to a limited extent, it is important to consider all of the possible hazards. Factors which affect the ability of the organism to survive etc. will include issues such as:

- whether the modified plant can occupy a wider niche than its unmodified parent, eg: because of greater salt tolerance, herbicide resistance;
- whether the modified organism can form survival structures such as seeds and how far they could be dispersed. Particular attention should be paid to whether there has been a change in the seed dispersal or dormancy mechanism compared to the unmodified plant.

b. Adverse effects on animals

This should include a consideration of toxicity and allergenicity. For example, a modified plant being used to produce pharmaceuticals for human use may be immunogenic for exposed animal populations; gene products also could be toxic to other organisms.

c. Ability to cause harm to plants (weediness of transgenic plant)

The characteristics of the GM plant which affect colonization, symbiosis and competition etc., should be considered.

d. Potential for transfer of genetic material between GMO and other organisms

Consideration should be given to whether gene transfer could take place and also the mechanisms by which transfer could occur. For plants, the dispersal by pollen could be relevant if it could contribute to the undesirable spread of genetic material between the GM plant and other organisms. If, for example, a plant may be modified to express a toxic gene in the roots to kill or deter boring insect larvae, and if this gene is transferred and expressed in other plants, it may have a deleterious effect on beneficial or non-target insects as well. In this regard it is important

to identify the local species with which the GM plant could introgress.

e. Products of gene expression, particularly if they are toxic

A GMO which has the potential to cause negative effects on other micro-organisms as a result of an inserted gene coding for toxic or allergenic product will pose a hazard. For example, the gene product might kill and reduce populations of target and non-target flora and fauna; in such a case, the hazard will be affected by the level and location of expression and activity of the gene product.

f. Phenotypic and genetic stability

The loss of a gene inserted into the GMO is unlikely to pose a hazard. However, genetic instability which leads to incorporation of genes elsewhere in the genome of the same GMO may be hazardous, producing, for example, variability in expression. Genetic instability may, therefore, give rise to phenotypic instability and consideration should be given to any possible detrimental effects that this could cause, eg: increase in levels of naturally occurring toxins.

g. Hazards due to cloning of genes from plant pests into transgenic plants

Where plant viral inserts are incorporated into transgenic plants, consideration should be given to the possibility of harm arising from the interaction of the insert and plant viruses. The following should be considered:

- **Transcapsidation:** is the formation of virus particles comprising of the genomic nucleic acid of one virus and the coat protein of another. Alternatively, a mixture of its own coat protein subunits and those of another virus could occur. In both cases the novel virus may have altered transmissibility, specificity and stability.

- **Recombination**: is potentially the most serious issue as results may be permanent. However, currently there is no hard evidence to show that recombination occurs at high levels in transgenic plants.

- **Virulence and mutability:-** where satellite systems are used to protect plants against viral infection, hazards may arise through interaction with infecting plant viruses. Consideration should be given to the possibility that a mild satellite, derived from a cDNA insert, may mutate causing virulence. The inserted transgene may cause synergestic effects when the transgenic plant is exposed to other viruses, and this should be considered in risk assessments.

h. Any other negative effects on organisms

(ii) Assessment of Likelihood of Risk

Likelihood of risk should be expressed as "high", "medium", "low" or "negligible" as described in Chapter 2. In addition to the factors described in Chapter 2, the following factors may also be considered where relevant:

a. ***Characteristics of the receiving local environment*** : climatic - geographical, and soil conditions, and the types of flora and fauna may be key factors.

b. ***Type of pollen dispersal method***: if dispersal is entirely by wind, likely dispersal distance may be low from a glasshouse. Where insect vectors are the main route of dispersal, pollination ranges should be considered. Consideration should include the number of organisms that might escape, small numbers being less likely to establish in the environment.

c. ***Transcapsidation***: where plants contain a transgene expressing a viral coat protein, the likelihood of transcapsidation should be considered. Plants can be infected with two or more viruses (commonly of the same genus), and in such plants transcapsidation could occur (see section 4.1.1.1.g). The likelihood of transcapsidation could be reduced by the use of mutated coat protein constructs.

(iii) Assessment of Consequence of Risk

Follow the guidelines given in Chapter 2. In addition, the following descriptions may help with regard to activities involving plants:

a. ***Severe consequence*** - a major change in the number of one or more species leading to negative effects on the functioning of the ecosystem(s).

b. ***Negligible consequence*** - no measurable change in population e.g. plant, animal, microbial, in the environment or in any ecosystem function.

(iv) Management of Risk

Follow the guidelines given in Chapter 2.

4.1.1.2 Risk Assessment for Human Health and Safety

The most likely hazards of GM plants will be allergenicity, toxicity, alteration in nutritive value, and antibiotic resistance. The possibility of accidental/incidental consumption should be considered. It is advisable to concentrate on hazards which result from the modification, rather than hazards which are present even in the non-modified plants.

4.1.1.3 Assignment of Final Containment and Control Requirements

The final containment and control measures assigned will depend on the nature and degree of risk to both the environment and humans. Control must be sufficient to reduce all risks to low or effectively zero, and thereby prevent harm from occurring (Follow the guidelines given in Chapter 2 for GMMs).

4.1.2.0 Containment

The following guidance sets out the specific control measures to contain GM plants. For activities involving GM plants infected/associated with GMMs, the "investigator " should also follow the recommendations given in Chapter 2. For additional guidance on bio-safety practices, see Chapter 5.

Some of the general principles of good practice as relevant to GM plants include:

- Training of personnel and provision of written standard operating procedures, and maintenance of records
- Maintenance of high standards of cleanliness
- Availability of washing and decontamination procedures
- Provision of suitable protective clothing

- Prohibiting eating, drinking, smoking, applying cosmetics, storing of food, and mouth pipetting
- Safe storage of all viable GM material and suitable disposal systems
- Suitable pest and disease control measures

4.1.2.1 Plant growth facilities

Plant growth facilities will vary in type from relatively impermanent structures (such as polytunnels), to permanent structures such as greenhouses and growth rooms. However, only permanent structures are recommended for GM plants. Two levels of containment are recognized: Containment Level A is recommended for work with plants that are unlikely to cause environmental harm and Containment Level B for work where harm could arise if the GM plant or its descendents were able to enter the environment.

(i) Containment Level A

This level includes activities where the risk is assessed to be negligible or low, and where the plant or control measures have one or more of the following features:

- The plant is incapable of surviving outdoors in Sri Lanka;
- The plant has limited ability to transfer genetic material to other Sri Lankan plant species;
- In the case of plants transformed using a plant pest which was a disarmed strain and which was subsequently removed during the plant regeneration procedure.

a. .Minimum Containment Measures for Level A:

In addition to the general principles of good practice outlined above, the following would normally be seen as constituting the basic level of containment for work with GM plants. However, the containment and control measures assigned must be determined by the risk assessment on a case by case basis.

Plant growth facility shall be a permanent structure and could be a separate building (greenhouse) or a clearly demarcated isolated area (growth-room) within a building. It should be properly maintained so that it will withstand normal climatic conditions within the period of activity. Windows should be closed and sealed. Access doors to the facility should be self-closing. Access to facility should be controlled and it should be kept locked when unattended. Hygeine facilities, including handwash basins should be available, ideally within the facility. The facility should be easy

to clean and should be maintained in a tidy condition. The use of floor drains should be avoided to minimise seed dispersal and invertebrate/vertebrate ingress. Experimental materials, including soil and other growth media, should be clearly marked and treated before disposal to kill any residual organisms/plant parts.

(ii) Containment Level B

This level should be used where the risk assessment identifies a hazard to humans and the environment, and the risks cannot be reduced to an acceptable level by the basic containment facilities (Level A). The activities in this category would include:

- GM plants with ability to transfer novel genetic material to Sri Lankan plant species;
- GM plants that could survive outside the containment facility and which could cause environmental harm;
- GM plants expressing plant pest derived sequences;
- GM plants which express hazardous substances as a result of modification;
- Work with GMMs and plant pathogens may be conducted on a case by case basis by adopting appropriate additional control measures listed in Chapters 2 and 5.

a. Minimum Containment Measures for Level B

In addition to the requirements of the general principles of good practice and containment A, the following measures should be implemented as a minimum:

Access to the facility to be limited to named personnel. Procedures for transfer of material between the greenhouse/growth room and other parts of the site, such as laboratories should be implemented to prevent dissemination of viable GM material. Tools, pots and other equipment in the facility should be treated after use in order to prevent dispersal. Disposable items should be stored securely pending final inactivation and disposal. Protective clothing may need to be cleaned/treated before removal from the facility, depending on the risk. In other cases, it may be sufficient to bag the clothing and send it for appropriate cleaning. An autoclave should be available in close proximity. The need to maintain negative pressure should be considered during construction. The walls, floors and ceilings of the greenhouse must be constructed to form a sealed internal shell that facilitates fumigation and must be animal proof especially against arthropods. Bench tops and other work surfaces should have seamless surfaces impervious to water and resistant to alkali, acids, organic solvents and moderate heat.

Additional control measures may be required for particular experiments (on a case by case basis) including activities involving GMMs and plant pathogens, and where the risk assessment has identified a particular route of exposure to the environment. Control measures for such experiments may include entry to the facility via a separated room with interlocking doors, the use of inward air-flows and the filtration of exhaust air. Operations under negative pressure, treatment of all effluent, availability of an autoclave on site and the use of laundry facilities on site may be appropriate. Work with plant pathogens should be carried out in separate areas, away from other plants. Separate compartments of large greenhouses may provide adequate separation.

4.1.3.0 Notification requirements

Approval needed for all activities on GM plants and products as described in section 2.4.0.0 (Chapter 2). The current regulations set out by the Plant Protection Ordiance are also applicable to all activities on GM plants.

4.2.0.0 GENETIC MANIPULATION OF ANIMALS

This provides additional guidance on the risk assessment activities involving GM animals. The term animal is used in its broadest sense, and includes both vertebrates and invertebrates. Activities involving both the risks from the GMMs (see Chapter 2) including animal viruses (Chapter 3) and the GM animals, the risks should be assessed together, to ensure that all aspects are appropriately controlled. Investigators should refer to Animals Act, Animal Disease Act, Animal Feed Act and Fauna and Flora Protection Ordinance for relevant information.

4.2.1.0 Additional Guidelines for Risk Assessment:

A risk assessment must be carried out and cover the following aspects:

(i) environment protection (including hazard identification, assessment of likelihood and consequences, assignment of risk management measures to protect the environment) and

(ii) human health and safety issues.

(iii) The final containment and control requirements will be assigned by adjusting (i) to take account of (ii).

4.2.1.1 Risk Assessment for Protection of the Environment

The aim is to determine whether the GM animal, or its descendants, could cause harm to the environment if it escapes, and appropriate containment and control measures are provided to prevent such harm occurring. The appropriate containment will vary with both the level of risk and the nature of the animal being controlled.

(i) Hazard Identification

a. ***Effect on biodiversity***: Capacity to survive, establish, disseminate, compete with and/or displace other animals.

The capacity of the GM animal to survive in the environment will be a key factor to consider. If the animal cannot survive outside of containment, it is unlikely to cause environmental harm. For example, nude mice are not likely survive outside of containment. Where a GM animal has an intrinsic ability to survive, it is important to consider how they might interact with the environment and whether they present any of the hazards listed below.

b. ***Adverse effects on animals and plants.***

GM animals may cause harm simply by their presence in the environment. If they are intrinsically adapted to the climate and environment,

- They may out number and displace other populations of animals;
- Predation may be a problem;
- Effects on plant species should be considered: establishment of significant populations of escaped GMOs may lead to overgrazing and serious ecological impacts. Loss of biodiversity in plant species may in turn affect the ability of the environment to support its normal animal populations.

c. ***Potential for transfer of genetic material between GMOs and other organisms.***

Consideration should be given to the possibility of the modified genetic material being transferred to wild or domestic relatives. Factors to consider will include:

- Presence of sexually compatible species in the environment;
- Whether the GM is sterile;
- The sex of the GM animal. If only females escape (and they are recallable) the chances of gene transfer into the wider population will be greatly reduced.

d. Products of gene expression, particularly if they are toxic.

It is relatively unlikely that animals will be modified to produce toxins *per se,* or that the modification will have pleotropic effects leading to alteration in natural toxin production. However, the possibility should be considered. It is more likely that animals will be modified to produce biologically active substances. The possible harmful effects of these active substances should also be considered.

e. Phenotypic and genetic stability.

Genetic instability is most likely to result in loss of introduced trait and is therefore unlikely to pose any harm. However, if the genetic modification itself is used as a biological control method (eg: sterility introduced by genetic modification) then the reversal of modification would reduce the level of control. Similarly, if a nutrient dependency introduced by modification is lost by genetic instability, there could be an increase in the possibility of dissemination of GM animals in the environment.

f. Any other negative effects on organisms

One example is the possibility of the GM animal acting as a novel animal disease vector.

(ii) Assessment of Likelihood of Risk

Likelihood of risk should be expressed as "high", "medium", "low" or "negligible" as described in Chapter 2. One of the key factors to be considered here is whether the GM animals

are contained so that their access to the environment is limited. It is suggested that the "investigator" starts by assuming the basic level of containment that would be applied to the equivalent non-GM animal. The basic level can then be revised as necessary until all the risks are reduced to "low" or "effectively zero". Other factors which might influence the likelihood of risk include:

- The surrounding environment and climatic condition. Could they support the short or long term survival, establishment and dissemination of the escaped GM animal? For example, a GM salmon next to coastal waters would have a high probability of establishing novel populations if they escape;
- Are the natural fauna and flora susceptible to disturbance by the GM animal?
- Where there are sexually compatible relatives there may be greater potential for gene transfer;
- Is it possible to recall escapees?
- Do the GM animals have an increased tendency to explore?

(iii) Assessment of Consequence of Risk

Follow the guidelines given in Chapter 2. In addition, with regard to GM animals, the following descriptions may help:

Severe consequence - a major change in the number of one or more species leading to negative effects on the functioning of the ecosystem(s).

Negligible consequence - no measurable change in population e.g. plant, animal, microbial, in the environment or in any ecosystem function.

(iv) Management of Risk

Follow the guidelines given in Chapter 2.

4.2.1.2 Risk Assessment for Human Health and Safety

The following should be considered.

(i) Risk of scratches, bites & allergenicity and zoonoses.
(ii) Risk from the genetic modification itself – allergenicity and toxicity.
(iii) Change in behaviour or physical nature – increased aggression.

(iv) Change in ability of animal to act as a human disease reservoir – e.g. insertion of a novel viral receptor.

4.2.1.3 Assignment of Final Containment and Control Requirements

The final containment and control measures assigned will depend on the nature and degree of risk to both the environment and humans. Control must be sufficient to reduce all risks to `**low**` or `**effectively zero**` and prevent harm from occurring.

4.2.2.0 Containment

The following guidance sets out the specific control measures to contain GM animals. For activities involving GM animals infected/associated with GMMs, the "investigator" should also take into account the recommendations given in section (Chapter 2). For additional guidance on bio-safety practices, see Chapter 5.

In the following guidance, animal containment is divided into three categories. Containment A represents the basic recommended level with good practice. Containment B represents a higher category of containment and Containment C activities involving GM animals infected with GMMs or other pathogens.

(i) Animal Containment Level A

Containment A is recommended for GM animals which exhibit any of the following properties:

- They are incapable of surviving in the environment in Sri Lanka;
- They have limited ability to transfer genetic material to Sri Lankan animal species;
- Female farm animals which are easily recalled;
- The genetic modification does not increase the level of risk to human health or the environment above that of the non modified parental organism.

a. Minimum measures for Containment A

Animals should be kept in appropriate containment, such as in animal rooms, or securely fenced areas, to minimise the possibility of accidental escape or theft. All potential routes of escape should be identified and appropriate measures put in place to prevent egress. The containment area should be kept locked where appropriate, and monitored at frequent intervals. Animal barriers should be placed on exits from animal rooms

to corridor areas when rooms or cages are being cleaned. A barrier should separate male and female animals unless reproductive studies are part of the experiment, or unless other measures are taken to avoid sexual reproduction. A written record should be maintained of the experimental use and disposal of each animal. Animals and cages should be appropriately labelled. Animals should be transported to and from the facility in appropriate animal containers. Access to the facility should be restricted. Staff should be given appropriate training and instructions on the procedures to be followed.

(ii) Animal Containment Level B

This level of containment is recommended for GM animals with one or more of the following characteristics.

- The animals could survive harm to humans or the environment if they escape from the containment facility, and they have the ability to transfer novel genetic material to Sri Lankan animal species;
- The animals could establish outside of the containment facility;
- The genetic modification increases the level of risk to human health and the environment above that of the non modified parental organism.

In all cases Containment B must be used when Containment A is insufficient to reduce all risks to "low" or "effectively zero".

a. Minimum Control Measures for Containment Level B

The following procedures and containment are recommended standards of good practice and should be applied in addition to the provisions of animal containment level A. The measures include additional barriers or more tightly controlled access. They will also need to be supplemented by measures for specific animal types. Where small animals are kept, floor drains should be avoided if possible. Written operating procedures should be produced for all routine operations, the staff should be trained appropriately, and written records of training should be maintained. Written records of any accidents or escapees from primary containment should be maintained. Access to the facility should be strictly restricted. Discharge of water from tanks holding aquatic animals must not be direct to drain. The disposal of carcasses from research facilities should be treated as clinical waste and handled appropriately.

(iii) Animal Containment Level C

This level of containment is recommended for activities involving GM animals infected with GMMs and pathogens. The "investigator" should also refer to Chapters 2 and 5 for additional control measures and guidance. The measures must be appropriate to control the risks to both humans and the environment. In addition to the requirements of levels A and B, the following measures should be adopted (as appropriate), on a case by case basis, to control and manage risks associated with each GM animal experiment involving GMMs/pathogens.

The facility must be isolated and doors must be lockable. It should be designed to be easily cleaned and decontaminated. An incinerator for disposal of animal carcasses must be accessible. There must be an autoclave on site. There must be appropriate barriers at room exit and on drains or ventilation duct work. Suitable protective clothing and gloves must be worn. All waste material contaminated with GMMs must be inactivated. If necessary, shower and sink effluent must be inactivated before final discharge. If the GMM is transmissible through an airborne route, HEPA filtration may be required. If the risk assessment indicates that it is necessary, a double ended autoclave must be used and personnel must have a complete change of clothing before entry and exit. Additional control measures must be adopted as required depending on the type of animal and on the activity.

4.2.3.0 Notification requirements

Approval needed for all activities on GM animals and products is as described in section 2.4.0.0 (Chapter 2).

CHAPTER 5

IMPLEMENTATION & RESPONSIBILITIES

5.1.0.0 MECHANISM FOR IMPLEMENTATION OF GUIDELINES

For implementation of the guidelines, it is necessary to have an institutional mechanism that would ensure the compliance of necessary safeguards at various levels of activity. The guidelines prescribe the safety procedures needed to be adopted for rDNA research, production and release of rDNA molecules to the environment and setting up of containment conditions for certain laboratory activities. Full compliance of these safeguards may be achieved by adopting a voluntary and regulatory approach. The proposed institutional mechanism and framework for implementation of guidelines is shown in Figure 5.1. The following are the regulatory/ implementation and advisory committees proposed to be formed:

(i) Institutional Biosafety Committee (IBSC).
(ii) Recombinant DNA Advisory Committee (RAC) (at national level)

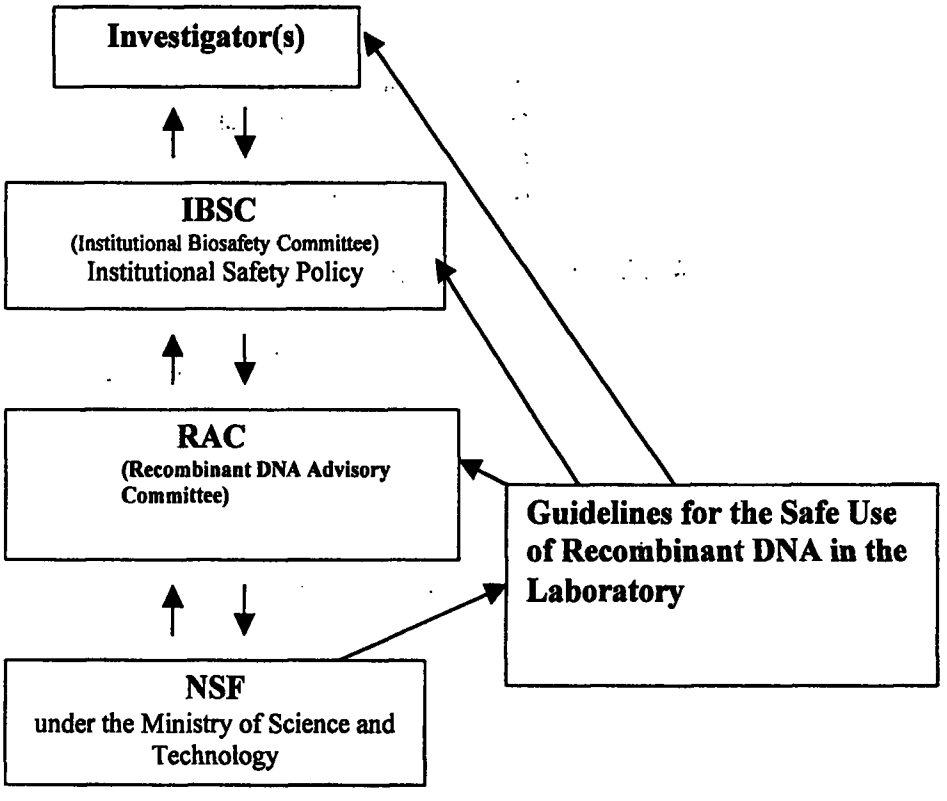

Figure 5.1

5.2.0.0 GENERAL RESPONSIBILITIES OF THE INVESTIGATOR(S)

On behalf of the institution, the investigator(s) is responsible for full compliance with the guidelines in the conduct of rDNA laboratory work.

The investigator shall:

(i) Make an initial determination of the required Containment Level for the work in accordance with the guidelines.

(ii) Seek approval from the IBSC prior to initiation or modification of rDNA work that requires consent from IBSC.

(iii) Notify the IBSC of all work, which requires IBSC notification simultaneous with initiation, and ensure that the appropriate safety procedures are followed.

(iv) Be adequately trained in good microbiological practices.

(v) Together with the Institutional Biosafety Officer, provide adequate training to staff and supervise the safety performance of the laboratory staff to ensure that safety practices are employed.

(vi) Adhere to IBSC approved emergency plans for handling accidental spills and personal contamination.

(vii) Provide information and possibly requests to RAC through IBSC to certify new vector/host systems.

5.3.0.0 SCOPE & FUNCTIONS OF ADVISORY AND IMPLEMENTATION COMMITTEES

5.3.1.0 Institutional Biosafety Committee (IBSC)

It is mandatory that all institutions conducting rDNA research establish an IBSC. The head of the institution shall be responsible for the establishment of this Committee. During the interim period of establishment of an IBSC, the Head of the institution may notify or seek approval for the proposed rDNA work from the RAC on behalf of the "investigator". The Institutional Biosafety Committee shall be the advisory body and the point of interaction within institutions for implementation of the guidelines. Any research project which is likely to be a potential biohazard (as envisaged by the guidelines) during the execution stage or which involves the production of either microorganisms or biologically active molecules that might cause biohazard should be notified to the IBSC. The IBSC will allow genetic engineering activity on classified organisms only at places where such work should be performed as per guidelines. Provision for suitable safe storage facility of donor, vectors, recipients and other materials involved in experimental work should be made and may be subjected to inspection on accountability.

The IBSC will constitute the following.

- Head of the institution or his/her nominee.
- Two members from the institution with proven experience in rDNA work or in molecular biology.
- One member from an outside institution who is an expert in rDNA work.
- Institutional Biosafety Officer – The institutional Head should appoint a Biosafety Officer and provide the necessary training.

5.3.1.1 Responsibilities of the IBSC:

(i) Documentation of all notified rDNA work carried out within the institution.
(ii) A list of all notified rDNA work performed within the institute should be sent to RAC each year.
(iii) Review and clearance of project proposals pertaining to the restricted category as outlined in the guidelines. No investigator should participate in reviewing their own project proposals on behalf of the IBSC. The IBSC would make every effort to issue clearance certificates quickly (within 1-2 months) on receiving the research proposals from investigators. A report on such decisions should be sent to the RAC under signature.
(iv) Preparation and adoption of an institutional laboratory policy on safety, in accordance with the guidelines.
(v) Training of personnel on biosafety.
(vi) Health monitoring program for laboratory personnel, utilizing existing medical facilities.
(vii) Regular monitoring of safety procedures in the institution.
(viii) Outlining of responsibilities of the biosafety officer depending on the institutional requirements.
(ix) Adopting emergency plans.
(x) Strict adherence to Intellectual Property Rights related confidentiality

5.3.2.0 Recombinant DNA Advisory Committee (RAC)

The RAC consists of a total of 10 members appointed by the National Science Foundation under the Ministry of Science and Technology. At least 8 members with proven experience in rDNA work representing medical/dental, veterinary, biology, agriculture, industry, chemistry and environment fields, have appointed. One representative each is appointed

from the Ministries of Science & Technology and Forestry & Environment.

5.3.2.1 Responsibilities of the RAC

(i) The committee would serve as the national advisory committee on rDNA work

(ii) Serve as the national focal point on rDNA activities and maintain records of all notified rDNA activities in the country.

(iii) Review and guide on activities relating to rDNA work as outlined in the safety regulations.

(iv) The Committee should study the developments at national and international levels in Biotechnology and make appropriate recommendations on current safety guidelines dealing with rDNA work.

(v) Review and update the list of certified vector/host systems.

(vi) Monitor safety facilities where rDNA work is being carried out and take appropriate action where necessary.

(vii) Recommend training programmes for technicians and research fellows, on the safe use of rDNA molecules in research.

(viii) Monitoring of activities of IBSCs.

(ix) Strict adherence to Intellectual Property Rights related confidentiality

CHAPTER 6

BIOSAFETY PRACTICES AND CONTAINMENT FACILITIES

6.1.0.0 BIOSAFETY PRACTICES WITHIN THE DIFFERENT TYPES OF CONTAINMENT FACILITIES

Three types of containment facilities are recognised for rDNA work involving microorganisms including viruses:

(i) Basic laboratory to handle Risk Groups (RG) 1 & 2 agents,
(ii) Containment laboratory for RG 3 agents
(iii) Maximum containment laboratory for RG 4 agents.

Note that while containment facilities (ii) and (iii) will require special safety practices, those cited for the basic laboratory should be adhered to as well.

6.1.1.0 The Basic Laboratory

The basic laboratory encompasses all laboratories working with RG 1 and 2 agents: those that present low or moderate risk to the laboratory worker and low or limited risk to the community. In some instances, particularly in clinical laboratories of hospitals, exposure to agents of high individual risk may occasionally or unexpectedly occur in the course of routine work. These possibilities must be recognised in developing safety plans and policies.

6.1.1.1 Code of Practice

This code is a listing of the most essential laboratory procedures, that are basic to safe laboratory practice. In many laboratories and national laboratory programmes, such a code may be given the status of "rules" for laboratory operation. In these guidelines various parts of the "code of practice" will be elaborated and explained.

It is emphasized that good laboratory practice is fundamental to laboratory safety and cannot be replaced by specialized equipment, which can only supplement it.

The most important rules are listed below, not necessarily in order of importance:

(i) Mouth pipetting should be prohibited.

(ii) Eating, drinking, smoking, storing food, and applying cosmetics should not be permitted in the laboratory work area. The laboratory should be kept neat, clean and free of materials not pertinent to the work.

(iii) Work surfaces should be decontaminated at least once a day and after any spillage of potentially dangerous material.

(iv) All laboratory workers should wash their hands after handling infectious materials and animals and when leaving the laboratory.

(v) All technical procedures should be performed in a way that minimises the creation of aerosols.

(vi) All contaminated liquid or solid material should be decontaminated before disposal or re-use; contaminated materials that are to be autoclaved or incinerated at a site away from the laboratory should be placed in durable leak-proof containers, which are sealed and labelled before being removed from the laboratory.

(vii) Laboratory coats, gowns, or uniforms should be worn in the laboratory; laboratory clothing should not be worn in non - laboratory areas; contaminated clothing should be disinfected by appropriate means.

(viii) Safety glasses, face shields, or other protective devices should be worn when necessary to protect the eyes and face from hazards.

(xi) Only persons who have been advised of the potential hazards and meet specific entry requirements (e.g. immunization) should be allowed to enter the laboratory working area; laboratory doors would be kept closed when work is in progress; access to animal houses should be restricted to authorized persons; children are not permitted in laboratory working areas.

(x) There should be an insect and rodent control programme. Animals not involved in the work being performed should not be permitted in the laboratory.

(xi) The use of hypodermic needles and syringes should be restricted to parenteral injection and aspiration of fluids from laboratory animals and diaphragm vaccine bottles. Hypodermic needles and syringes should not be used as a substitute for automatic pipetting devices in the manipulation of infectious fluids. Cannulas should be used instead of sharp needles wherever possible.

(xii) Gloves should be worn for all procedures that may involve accidental direct contact, with blood, infectious materials, or infected animals. Gloves should be autoclaved with other laboratory wastes before disposal. All spills, accidents and overt or potential exposure to infectious material should be immediately reported to the laboratory supervisor or the person assigned by the head of the division. A written record should be prepared and maintained. Appropriate medical evaluation, surveillance and treatment should be provided.

(xiii) Baseline serum samples may be collected from and stored for all laboratory staff at risk. Additional serum specimens may be collected periodically depending on the agents handled or the function of the facility.

(xiv) The investigator /laboratory supervisor (or a person assigned by the head of division) together with the Biosafety Officer should ensure that training in laboratory safety is provided. A safety manual (prepared by the IBSC) that identifies known and potential hazards and that specifies practices and procedures to minimise or eliminate such risks should be adopted. Personnel should be advised of special hazards and required to read and follow standard practices and procedures.

(xv) Space and facilities should be provided in the safe handling and storage of solvents, radioactive materials and compressed gases. Handling and storage of radioisotopes should be performed as per instructions given by the Atomic Energy Authority, Sri Lanka.

(xvi) Safety systems should cover fire, electrical emergencies, emergency shower, and eyewash facilities.

(xvii) First-aid areas or rooms suitably equipped and readily accessible, should be available.

(xviii) A good-quality and dependable water supply is essential. When designing new laboratories, there should be no cross connections between water sources for laboratory purposes and for drinking.

(xix) A reliable electricity supply with adequate capacity should be available. A standby generator with automatic cut-off is desirable for the support of essential equipment -incubators, freezers, etc.

(xxi) A reliable gas supply to each working area is essential. Good maintenance of the installation is mandatory.

(xxii) Three aspects of waste disposal need special attention (details of which are given in section 6.1.1.7) to meet performance and/or pollution control requirements.

(xxiii) Autoclaves and sterilizers for treatment of solid wastes may be needed for certain activities.

(xxiv) Liquid waste to be handled appropriately.

(xxv) Incinerators may be needed for the disposal of items of certain activities.

(xxvi) Restricted access to animal houses.

6.1.1.2 Laboratory equipment

The risk of an infection can be minimised by the use of safety laboratory equipment, practices and facilities. This section deals primarily with laboratory equipment suitable for work with RG 2 and 3 agents.

The head of the laboratory, after consultation with the investigators, Safety Officer and Safety Committee, should ensure that adequate equipment is provided and that they are used properly. In selecting safe laboratory equipment, in general, the following should be considered.

(i) Equipment

a. Designed to limit or prevent contact between the operator and the infectious agent;

b. Constructed from materials that are impermeable to liquids, corrosion-resistant, and meet structural strength requirements;

c. Fabricated to be free of burns and sharp edges;

d. Designed, constructed and installed to facilitate simple operation and to provide for ease of maintenance and accessibility for cleaning. Detailed performance and construction specifications may be

required to ensure that the equipment purchased will possess the necessary safety features.

(ii) Recommended Biosafety Equipment

a. Pipetting-aids to replace mouth pipetting. These are available in many designs.

b. Biological safety cabinets to be used whenever procedures with a high potential for creating hazardous aerosols are conducted. These may include centrifugation, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressure may be different from the ambient pressure, intranasal inoculation of animals, and harvesting infected tissues from animals or eggs. When high concentrations or large volumes of infectious agents are handled, the material may be centrifuged in the open laboratory only if sealed heads or centrifuge safety cups are used. They should only be opened in a biological safety cabinet.

c. Loop microincinerators to reduce aerosol production.

d. Screw-cap tubes and bottles to provide positive specimen containment.

e. Autoclaves to sterilize contaminated material.

6.1.1.3 Health and medical surveillance

The objectives of the health and medical surveillance of laboratory personnel are:

(i) To provide a means of preventing occupationally acquired disease

(ii) To assess the efficacy of protective equipment and procedures.

It is the responsibility of the head of the institution to ensure that health and medical surveillance of laboratory personnel is carried out.

6.1.1.4 Training

Human error and poor laboratory practice can compromise the best of laboratory safeguards and equipment provided specifically to protect the laboratory worker. Thus, a safety-conscious staff, well informed about the recognition and control of hazards present in the laboratory, is the key element in the prevention of laboratory accidents and acquired infections. For this reason, continuous on-the-job training in safety measures is essential. Safety measures should always be an integral part of a new employee's introduction to the laboratory. Training programmes should be incorporated into the institutional set-up.

Laboratory supervisors must play a key role in training their immediate staff in good laboratory practice. The institutional safety officer can assist in training.

6.1.1.5 Handling, Transfer and Shipment of Specimens

The handling, transfer and shipment of improperly packed specimens and infectious agents carry a risk of infection to the people directly engaged in, or in contact with, any part of the process. Improper handling within the laboratory endangers not only the immediate staff but also administrative, secretarial and other support personnel. Transfer of material between laboratories or institutions widens the scope of risk to the public and to airline and postal personnel.

(i) Internal handling procedures:

a. ***Specimen containers:*** Specimen containers should be leak-proof. No material should remain on the outside after the cap has been closed.

b. ***Transport:*** To avoid accidental leakage or spillage into the environment, special secondary containers should be provided for the transport of specimens between wards or departments and laboratories. These should be made of metal or plastic.

c. ***Reception of specimens:*** Where large numbers of specimens are received, a separate room should be provided for their receipt. In a small facility, this may be part of a laboratory room.

d. ***Opening of packages:*** Ideally, all packages containing infectious agents received via mail or airfreight or other common carrier should be opened in a

biological safety cabinet.

(ii) Shipment by Mail, Airfreight or Other Common Carrier

Infectious substances are classified as dangerous goods. Packages containing such substances must bear the infectious substance (biohazard) label (page 18 figure 2.3) and shipment procedures outlined by import and export control regulations should be followed. Investigators should refer to Agricultural Products Ordinance, Import and Export Control Act and Customs Ordinance for relevant information.

6.1.1.6 Emergency procedures

Emergency contingency plans should be prepared for each individual laboratory as well as for the institution. These are best prepared by the individual laboratory supervisor in conjunction with his staff and the safety officer. This procedure offers the best prospect of success as it is the immediate staff who is most familiar with the hazards associated with the particular laboratory.

Once the emergency plan is formulated, it should be pasted in a conspicuous place in the laboratory for immediate reference.

Emergency plans should provide for:

(i) breakage and spillage,
note: each laboratory should follow appropriate procedures depending on the bench, floor purposes and the hazardous agent

(ii) accidental injection, cuts and abrasions,

(iii) accidental ingestion of potentially hazardous material,

(iv) a potentially hazardous aerosol release (other than in a safety cabinet),

(v) breakage of tubes in centrifuges not having safety cups,

(vi) fire, flood and natural disaster,

(vii) vandalism,

(viii) emergency services - whom to contact,

(ix) emergency equipment and its location.

6.1.1.7 Decontamination and disposal

Decontamination and disposal in laboratories are closely interrelated acts, since disinfection or sterilisation constitutes the first phase of disposal. All material and equipment will ultimately be disposed. However, in terms of daily use, only a portion of these will require actual removal from the laboratory for disposal. The remainder will be recycled for use within the laboratory, examples being re-usable laboratory glassware, instruments and laboratory clothing.

The principal questions to be answered prior to disposal of any objects or material from laboratories dealing with potentially infectious microorganisms or animal tissues are:

Have the objects or material been effectively disinfected or sterilised according to an approved procedure?

If not, have the objects or material been packaged in an approved manner for immediate on-site incineration or transfer to another laboratory?

Does disposal of the disinfected or sterilised objects or materials involve any additional potential hazards, biological or otherwise, to those carrying out the immediate procedure or those who might come into contact with the objects or material outside the laboratory complex?

(i) Decontamination

Autoclaving is the procedure of choice for all decontamination processes. The autoclave should be of the gravity displacement type and worked upon at 1.4 kg/cm^2 pressure for 30 minutes. Some procedures may not require autoclaving for decontamination – in such events:

(a) boiling for 30 minutes, preferably in water containing sodium bicarbonate,

(b) use of a pressure cooker at the highest attainable working pressure.

(c) Use of disinfectants and chemicals: there should be a written disinfectant policy stating which disinfectants are listed for which purpose and its dilution for each.

Sodium hypochlorite and formaldehyde are the disinfectants recommended for general laboratory use.

For special purpose, phenolic compounds, various surface-active and/or lipid-destroying agents, including alcohols, iodine and iodophors and other oxidizing agents, as well as very high or extremely low pH, can be effective, provided that it has been established that the agent to be destroyed is not resistant to the procedure.

(ii) Disposal

An identification and separation system for contaminated materials (and their containers) should be established.

a. Non-contaminated waste: can be disposed of with general waste,

b. "sharps", needles, syringes, etc.,: Hypodermic needles and disposable syringes should be placed in containers with walls that are not readily penetrable. When full, these should be placed in contaminated waste containers and incinerated, even if laboratory practice requires that they are autoclaved first.

c. Contaminated material for autoclaving and recycling: The material is placed in shallow leakproof containers containing enough of a suitable disinfectant to cover the contents. The containers are then placed in the autoclave. No pre-cleaning is performed; and necessary cleaning or repair is done after autoclaving.

d. Contaminated material for disposal: All cultures and contaminated material are normally autoclaved in leakproof containers prior to disposal. Following autoclaving, the material may be placed in transfer containers for transport to the incinerator or other point of disposal.

In some situations, the autoclaving step is not required. In such instances, the contaminated waste is placed in specially marked containers and transported directly to an incinerator. The best practice is to place a plastic bag for containing the waste in a paperboard box; then the contents

and container can all be incinerated. If transfer containers are used, they should be cleaned and disinfected after emptying the contaminated waste and prior to return to the laboratory. Such containers should be leakproof with tight-fitting covers.

Incineration is the method of choice for final disposal of contaminated waste, including carcasses of laboratory animals. Incineration for this purpose must meet with the approval of public health and air pollution authorities and the safety officer.

Where incinerators are not approved for such use, final disposal methods must be established in cooperation with public health authorities.

6.1.1.8 Animal facilities

The use of laboratory animals for experimental and diagnostic purposes imposes on the user the obligation to take every care to avoid causing the animals unnecessary pain or suffering. They must be provided with comfortable, hygienic housing and adequate, wholesome food and water. At the end of the experiment they should be destroyed in a humane manner.

The animal house or room should be an independent, detached unit. If it adjoins the laboratory facilities, the design should provide for its isolation from the public laboratory should such needs arise.

The design and layout of the unit will vary greatly depending on the species of animals to be accommodated, the nature of the work programme, and local climatic conditions. Individual rooms are required to separate animals according to the degree of hazard of the agents under investigation. Additional design requirements may be obtained from publications devoted to laboratory animal care.

6.1.1.9 Chemical, Electrical, Fire, and Radiation Safety

A breakdown in the containment of pathogenic organisms may result indirectly owing to fire or chemical, electrical, or radiation accidents. It is, therefore, mandatory to maintain high standards of chemical, electrical, fire and radiation safety in the microbiology laboratory.

Statutory rules and regulations for each of these laid down by the competent national or local authorities apply (eg. for radiation safety - Atomic Energy Authority). Their assistance and guidance should be sought if necessary.

6.1.2.0 The Containment Laboratory

The containment laboratory is designed and provided for work with RG 3 agents - those that present a high risk to laboratory workers but a low risk to the community.

This level of containment requires strengthening of the basic laboratory operational and safety programmes as well as the provision of added structural safeguards and the mandatory use of biological safety cabinets.

The guidelines are presented in the form of modifications of the guidelines for the basic laboratory. Therefore, the reader must first apply the basic laboratory guidelines, before those specific for containment laboratories. The major changes are in:

(i) The code of practice
(ii) Laboratory design and facilities
(iii) Health and medical surveillance

6.1.2.1 Code of practice

The code of practice for a basic laboratory applies, except where modified as follows:

(i) The two-person rule should apply, whereby no individual works alone within the laboratory.

(ii) A hazard warning sign should be displayed on laboratory doors, identifying the agent, the name of the laboratory supervisor and other responsible person(s) and indicating any special conditions of entry into the area (immunisation, etc.)

(iii) Laboratory clothing that protect street clothing must be worn. Laboratory clothing must not be worn outside the laboratory and must be decontaminated before being laundered all laboratory clothing and utensils must not be shared.

(iv) When appropriate, respiratory protective equipment should be worn in rooms containing infected animals.

6.1.2.2 Laboratory equipment

The use of a Class 111 biological safety cabinet or a flexible-film isolator may be indicated for procedures with RG 3 micro-organisms.

6.1.2.3 Laboratory design and facilities

The containment laboratory is designed for work with RG 3 agents and with large volumes and high concentrations of RG 2 agents, where there is a high risk of aerosol spread or infection.

The section on design and facilities for a basic laboratory applies (6.1.1.0), except where modified as below:

(i) The laboratory should be separated from areas that are open to unrestricted traffic flow within the building. A double-door system should be in place where entry to the laboratory is through an ante-room.

(ii) The surfaces of walls, floors and ceilings should be water-resistant and easy to clean. Openings in these surfaces should be sealed.

(iii) A foot or elbow-operated wash-hand basin is recommended near each laboratory exit door.

(iv) Windows in the laboratory should be closed.

(v) Access doors to the laboratory should be self-closing and lockable.

(vi) An autoclave for decontamination of laboratory wastes should be available within the laboratory. If infectious wastes have to be removed to another area in the same building for disinfections, they should be held and transported in a covered, leakproof container.

(vii) A ventilation system that establishes a negative pressure into the laboratory or an exhaust system where the air is not recirculated to other areas of the building, but within the laboratory should be installed.

(viii) The HEPA-filtered exhaust air from Class 1 or Class 11 biological safety cabinets should be discharged directly to the outside or through the building exhaust system. (HEPA: high-efficiency particulate air).

6.1.2.4 Health and Medical Surveillance

The objectives of health and medical surveillance programmes for basic laboratories apply to containment laboratories, except where modified as follows:

(i) Medical examination of all laboratory personnel working in the containment laboratory is mandatory. This examination should include a detailed past medical history and clinical examination.

(ii) A baseline serum sample should be obtained and stored for future reference.

(iii) Employees being treated with immunosuppressive drugs should not be employed in containment laboratories.

6.2.0.0 Maximum Containment Laboratory

The maximum containment laboratory is designed for work with infectious agents or experiments in microbiology that present, or are suspected to present, a high risk to both the laboratory worker and the community.

Construction and operation of a maximum containment laboratory should be preceded by intensive consultations with institutions that have experience in operating a maximum containment laboratory.

Operational maximum containment laboratories should be under the control of national or other appropriate health authorities.

The principal features of a maximum containment laboratory are:

(i) **Controlled access**: entry and exit of personnel and supplies are through airlock systems. On entering, personnel put on a complete change of clothes and they shower on exit before putting on their street clothing.

(ii) **Controlled air system**: Negative pressure is maintained by an individual supply and exhaust air mechanical ventilation system with HEPA filters in the exhaust, (and in the intake when necessary).

(iii) **Decontamination of effluents**: All effluents from the maximum containment laboratory are to be rendered safe, including the shower water.

(iv) **Sterilization of waste and materials**: A double-door pass-through autoclave is provided.

(v) **Primary containment**: An efficient primary containment will consist of one or more of the following: (a) Class III biological safety cabinet, (b) flexible-film isolators to similar standards, and (c) a positive-pressure ventilated suit. In this case, a special decontamination shower must be provided for personnel leaving the suit area.

Because of the great complexity of the work, a detailed work manual should be developed and tried out in training runs.

In addition, an effective emergency programme must be devised. In the preparation of this programme active cooperation with national and local health authorities should be established. Other emergency services, e.g. fire, police, receiving hospitals, should likewise be involved.

APPENDIX I

Classification of micro-organisms on the basis of Risk Groups

Micro-organisms are classified according to the risks posed by them to the handlers, and the ease of their transmission to the society. In the following classification, certain microorganisms have been classified at a higher or lower risk category depending on the conditions prevalent in the country. For example, Foot and Mouth Disease virus (attenuated strain) has been assigned to a lower Risk Group since the virus(es) are widely prevalent in the country. Similarly, the other pathogens widely prevalent in the country are brought under a lower category of Risk Group. Some of the microorganisms not present in the country have been assigned to a special category requiring the highest degree of safety, for example - Lassa virus, Yellow fever virus, etc.

Bacterial	
Risk Group 1	All bacterial agents not included in higher classes according to "Basis for Agent classification".
Risk Group 2	*Actinobacillus* - all species except *A. mallei,* which is in Risk Group 3. *Arizona hinshawii* - all serotypes *Bacillus anthracis* **Bordetella* - all species *Borrelia recurrentis, B. vincenti* *** Clostridium. C. chauvoei, C. difficile, C. fallax, C. haemolyticum, C. histolyticum, C. novyi, C. perfringes, C. septicum, C. sordelbi* *Corynebacterium diptheriae*,C. equi, C. haemolyticum* *C. pseudotuberculosis, Areanobacterium pyogenes, C. renale* *Diplococcus* (Streptococcus) *pneumoniae* *Erysipelothrix insidiosa* *Escherichia coli* - all enteropathogenic serotypes *Haemophilus ducrevi, H. influenzae, H. pneumoniae* *Herellea vaginicola* *Klebsiella* - all species and all serotypes *Letionella* *Leptospira interrogans* - all serotypes reported in Sri Lanka *Listeria* - all species

* Cloning agents and strains for human vaccine production.

** Agents likely to be employed for recombinant work in the veterinary field.

Risk Group 2 Contd.	*Mima polymorpha* *Moraxella* - all species *Mycobacteria* - all species including *Mycobacterium avium, M. bovis, , M. tubecurclosis, M. leprae* ***M. tuberculosis*** *, *M. leprae** ***Mycoplasma* - all species except *M. mycoides* and *M. agalactiae* *Neisseria gonorrhoeae, N. meningitidis** *Pasteurella* all species including *Pasteurella multocida* type B ("buffalo" and other foreign virulent strains) **Salmonella* all species and all serotypes*** **Shigella* - all species and all serotypes *Sphaerophorus neorophorus* *Staphylococcus aureus* *Streptobacillus moniliformis* *Streptococcus pyogenes, S. equi, S. pneumonine** *Streptomyces madurae, S. pelleteri, S. somaliensis* *Treponema carateum, T. pallidum* and T *pertenue* * *Vibrio foetus, V. comma* including biotype EIT or and *V. parahemolyticus.* *Vibrio cholerae*
Risk Group 3	*Actinobacillus mallei* *Bartonella* - all species *Brucella* - all species *Clostridium botulium, C. tetani** *Francisella tularensis* .Drug resistant *M.tuberculosis* *Pseudomonas Pseudomallai* *Yersinia pestis*

Fungal	
Risk Group 1	All fungal agents not included in higher classes according to "Basis for Agent Classification"

* Cloning agents and strains for human vaccine production.

** Agents likely to be employed for recombinant work in the veterinary field.

Risk Group 2	*Actinomycetes* (including) *Nocardia* and *Actinomyces* species and *Arachina propionica* *Aspergillus fumigatus* *Blastomyces dermatitidis* *Cryptococus neoformans, C. fersiminosos* *Epidermophyton madurella, E. microsporon* *Paracoccidioides brasiliensis* *(Sporothrix* *Trichoderma* Trichophyton)
Risk Group 3	*Coccidioides immitis* *Histoplasma capsulatum* Histoplasma capsulatum var. duboissi*

Parasitic	
Risk Group 1	All Parasitic agents not included in higher classes according to "Basis for Agent Classifications"
Risk Group 2	**Entamoeba histolytica* **Leishmania species* *Naegleria gruberia* *Plasmodium thcilera* *Plasmodium fabesia, P. falciparum* *Schistosoma* *Toxoplasma gondii* *Toxocara canis* *Trichinella spiralis* *Trichomonas* *Trypanasoma cruzii*
Risk Group 3	*Schisistosoma**. *S. mansoni*

* Cloning agents and strains for human vaccine production.

** Agents likely to be employed for recombinant work in the veterinary field.

Viral, Rickettsial and Chlamydial	
Risk Group 1	All viral, rickettsial and chlamydial agents not included in higher classes. In addition the following too are included: Influenza virus A/PR8/34 **New castle disease virus – strains licensed for vaccine use Parainfluenza Virus 3, SF4 strain **Rinderpest – attenuated virus strain (e.g. Kobatte-O) licensed for vaccine use.
Risk Group 2	Adenoviruses – Human, all types Avian loukosis Cache Valley virus CELO (avian adenovirus) Coxsackio A and B viruses Corona viruses Cytomegalo viruses Dengue virus Echo viruses – all types Encephalomyocarditis virus (EMC) Epstein – Barr virus Flanders virus* Hart Part virus *Hepatitis-associated antigen material – hepatitis A and B viruses, non A and non B, hepatitis C, HDV Herpes viruses – except Herpes virus simiae (monkey B virus) which is in Risk Group 4. Herpes simplex 2 Infectious bronchitus** Infectious Bovine Rhinotraechitis virus (IBR) Infectious Bursal diseases of poultry **Infectious Laryngotraechitis (ILT) *Influenza virus – all types, except A/PR8/34 which is in Risk roup I Langat virus eucosis complex** Lymphogranuloma venereum agent **Marek's Disease virus *Measles virus

* Cloning agents and strains for human vaccine production.

** Agents likely to be employed for recombinant work in the veterinary field.

	Mumps virus **Newcastle disease virus (other than licensed strain for vaccine use) Parainfluenza viruses – all types except Parainfluenza virus 3, SF4 strain, which is in Risk Group I *Polio viruses – all types, wild and attenuated Poxviruses – all types except Alastrim, monkey pox, sheep pox and white pox which, depending on experiments, are in Risk Group 3 or 4. **Rabies virus – all strains except rabies street virus, which should be classified in Risk Group III when inoculated into cornivores Reoviruses – all types Respiratory syncytial virus Rhinoviruses – all types Rinderpest (other then vaccine strin in use) Rubella virus Simian viruses – all types except herpes virus simiae (Monkey B Virus) which is In Risk Group IV Simian virus 40 Ad 7 SV 40 (defective) Sindbis virus Tensaw virus Turlock virus Vaccinia virus Varicella virus Vole rickettsia Yellow fever virus, 17D vaccine strain[2]
Risk Group 3	African Horse Sickness (Attenuated strain except animal passage) Alastrim, monkey pox and whitepox, when used *in vitro* Arboviues – *All strains except those in Risk Group II and IV Blue Tongue virus (only serotypes reported in Sri Lanka) Feline Leukemia Feline sarcoma **Foot-and-Mouth Disease virus (all serotypes and subtypes) Gibbon Ape Lymphosarcoma Herpes virus ateles Herpes virus saimiri HIV-1 & HIV-2 and strains of SIV Infectious Equine Anaemia

* Cloning agents and strains for human vaccine production.
** Agents likely to be employed for recombinant work in the veterinary field.

	Lymphocytic choriomeningitis virus (LCM) Psittacosis-ornithosis-trachnoma group of agents Pseudorabies virus Rabies street virus, when used in inoculations of carnivores Rickettsia – all species except Vole rickettsia and *Coxiella burnetti* when used for Vector transmission. **Sheep pox (field strain) Swine Fever virus Vesicular stomatitis virus (VSU) Woolly monkey Fibrosarcoma Yaba pox virus Non-defective Adeno-2 SV-40 hybrids
Risk Group 4	Alastrim, monkeypox, whitepox, when used for transmission or animal inoculation experiments Hemorrhagic fever agents, including Crimean hemorrhagic and Korean hemorrhagic fever (Congo) and others as yet undifined. Herpes virus simae (money B virus) Tick-borne encephalitis virus complex, including Russian Spring Summer Encephalitis, Kyasanur Forest Disease, Omsk hemorrhagic fever and Central European Encephalitis viruses

Special category Bacterial	
	Contagious Equine Metritis (H. equigenitalis) Pestis petit de ruminantium

Special category - Viral Rickettsial and Chlamydial	
	African Horse Sickness virus (serotypes not reported in Sri Lanka an challenge strains) African Swine Fever Bat rabies virus Blue tongue virus (serotypes not reported in Sri Lanka) Exotic FMD virus types and sub-types Junin and Machupo viruses Lassa virus Marurg virus Murrey valley encephalities virus Rift Valley Fever virus Smallpox virus-Archieval storage andpropagation Swine Vesicular Disease Venezeulan equine encephalitis virus – epidemic strains Western Equine encephalitis virus **Yellow fever virus – Wild strain Other arboviruses causing epizootics and so far not recorded in Sri Lanka*

* Cloning agents and strains for human vaccine production.
** Agents likely to be employed for recombinant work in the veterinary field.

APPENDIX II

Table 1. Access factors for host/Vector combinations

Vector **	Host *		
	Especially Disabled [A]	Disabled or non colonising[B]	Pathogenic colonising or Wild type[C]
Non-mobilisable[1]	10^{-12}	10^{-9}	10^{-3}or 1
Mobilisation-defective[2]	10^{-9}	10^{-6}	10^{-3}or 1
Self mobilisable[3]	10^{-6}	10^{-3}	1

* Host

[A] means one whose growth requires the addition of specific nutrients not available in humans or outside of the culture media and is sensitive to physical conditions or chemical agents present in man or the environment. This definition applies to certain specific organisms with an extended history of safe use, as well as some strains of *E.coli* K12 and cell or tissue culture systems where the vector does not have the ability to infect or transfer DNA to other cells **(Appendix III).**

[B] means a multiple auxotroph or other host which is unlikely to persist in the gut, lung, or survive outside of the culture media, e.g. most strains of *E.coli* K12 and other species **(Appendix III).**

[C] includes all other hosts. A value of 1 applied if it is pathogenic or non-pathogenic but able to colonise humans. A value of 10^{-3} is appropriate if it is wild type and capable of survival outside of culture **(Appendix III).**

**Vector

[1] are Bom$^-$, (Nic$^-$), Mob$^-$ and Tra$^-$, They include *E.coli* plasmid vectors such as pUC pAT153, pACYC184, pBR327 and pBR328 and their derivatives **(Appendix III).**

[2] are usually Bom$^+$ but Mob$^-$ and Tra$^-$. They include *E.coli* vectors such as pBR322, pBR325, RP4DI, pACYC177 and p15A and their derivatives **(Appendix III).**

[3] are Bom$^+$, Mob$^+$ and/or Tra$^+$. They include plasmid RP4, RSF1010, ColE1 & F **(Appendix III).**

Table 2. Relative values for the expression factor for an initial cloning experiment

Factor	Value
Deliberate in-frame insertion of expressible DNA downstream of a strong promoter (e.g. PL, PR Tac, trp, lac, Cu) with the intention of maximising expression (e.g. vectors pDS-5, pUC8-1, pUC9-1).	1
Insertion of expressible DNA downstream of a strong promoter (see above) with no attempt to maximise expression	10^{-3}
Insertion of expressible DNA into a site of limited promoter activity (e.g. Bla promoter in pBR322).	10^{-6}
Insertion of expressible DNA at a site specifically engineered to prevent expression (e.g. pDOC55, pNH series)	10^{-9}
Non-expressible DNA, e.g. DNA with no foreseeable biological effect or gene containing introns which the host is unable to process.	10^{-12}

Table 3. Recommended values for the damage factors

Factor	Value
A toxic substance or pathogenic determinant that is likely to have a significant biological effect.	1
A biologically active substance which might have a deleterious effect if delivered to a target tissue, or a biologically inactive form of a toxic substance which, if active, might have a significant biological effect.	10^{-3}
A biologically active substance which is very unlikely to have a deleterious effect or, for example where it could not approach the normal body level (e.g. less than 10% of the normal body level).	10^{-6}
A gene sequence where any biological effect is considered highly unlikely, either because of the known properties of the protein or because of the high levels encountered in nature.	10^{-9}
No foreseeable biological effect (e.g. non-coding DNA Sequence).	10^{-12}

Table 4. Provisional containment levels for human health

Overall value	Containment level
10^{-15} or lower	1
0^{-12} or lower	2
10^{-9} or lower	3
10^{-6} or lower	3 or 4 (case by case)
greater than 10^{-6}	4

Note: An indication of GMO's potential to cause harm to human health is obtained by multiplying the individual values allocated under access, expression and damage.

Table 5: Examples showing the assessment of the likelihood and consequence of hazard, and the degree of risk.

Example 1	Example 2
Consider the escape of a psuedomonad, isolated from soil but not disabled, which contains a promiscuous conjugative plasmid incorporating a gene expressing a bacteriotoxin to a wide range of soil-borne bacteria.	Consider the escape of a GM bacterial pathogen of the Grey Seal.
Hazard	
The potential for gene transfer would constitute a hazard, as would the expression of a gene for the toxic protein.	If it is unable to survive even for a short time in the environment, the potential for transfer of gene codings for pathogenic traits to other indigenous bacteria would be a hazard. If it is able to survive, the pathogenicity of the organism would be an additional hazard.
Likelihood of hazard	
The likelihood of exposure of GMM to the soil in the vicinity of the lab may be very low if proper containment is set. However, if a few GMM still escape the likelihood of gene transfer and of expression of the toxic would be "high"	The likelihood of hazard would depend on the place of work. If the lab is far removed from any water course, the escape of the GMM would result in a "negligible" likelihood since the potential receiving environment contained no access to marine or littoral environment. However, if the work is done in a coastal lab, there might be a "high" likelihood of hazard.
Consequence of hazard	
If the bacteriocin gene were expressed, the consequences might be "severe", "medium ", "low" or "negligible". For example: severe consequence - a major change in the number of one or more species leading to negative effects on the eco system. Negligible consequence - no measurable change in any microbial population in the environment	Any contact of the GMM with the host species is likely to cause a "severe" consequence.
Degree of risk	
The key factor influencing the likelihood of hazard is the level of containment set. Thus, even if the consequence of gene transfer or expression were "severe" the resultant risk might be anything from "high" (if containment were inadequate) to "effectively zero" (if containment were adequate).	The likelihood of hazard is dependent not only on containment but also on the location of the lab. The risk posed would be "severe" for a coastal lab and would be "effectively zero" for a inland lab.
Management of Risk	
If the level of containment is inadequate, the risk posed would be "medium" or above. Then, additional control measures should be taken to raise the containment level.	The containment level applied to work with the pathogen would need to be increased if risk were "medium" or above.

Table 6: Estimation of Risk[1]

Consequences x likelihood = risk of causing "harm"

Consequence of Hazard	Likelihood of Hazard			
	High	Medium	Low	Negligible
Severe	High	High	Medium/Low	Effectively Zero
Medium	High	Medium	Medium	Effectively Zero
Low	Medium/Low	Low	Low	Effectively Zero
Negligible	Effectively Zero	Effectively Zero	Effectively Zero	Effectively Zero

[1]This is not intended to be a definitive matrix for risk

APPENDIX III

A. Examples of Host/Vector Systems and Access Factor

This Annex lists some examples of disabled hosts of poorly mobilisable vectors and should be used in conjunction with Table 1 of Appendix II.
This Annex only intends to give "key" host/vector systems and does not aim to list all of the derivatives. In listing key systems, this guidance allows flexibility in the assignment of access factors to a particular host-vector system by the person undertaking the assessment.

Host systems

1. The assignment of factors for access in the main text depends on features of the host species and on the cloning vector. In Table 1 (Appendix II), hosts are divided into three classes; especially disabled, disabled or non-colonising or pathogenic, colonising or wild type depending on their ability to colonise or infect humans and to survive outside of the culture media.

2. Factors which limit colonisation, infection or survival are often termed biological barriers and can either be inherent physiological features of the organism or the result of mutations. In this sense, certain 'wild type' (i.e. not laboratory adapted or artificially mutated) hosts often have suitable biological limitations (eg inability to grow at 37^0C) and could equally well be considered to fall into the 'disabled' class of hosts for the purposes of a risk assessment with respect to human health and safety.

3. As well as the examples below, users may find it helpful to consider the guidance in Appendix I when determining if a host is non-pathogenic. This, together with the ability to survive or colonise humans, will determine whether the host may be eligible to be considered "disabled".

(i) Especially disabled hosts

4. This category of hosts has been defined as those which are non-pathogenic, are unlikely to survive outside of the culture media and have a history of safe use. This category is applied to only a few species of non-pathogenic micro-organisms and to certain well defined derivatives of acknowledged pathogens. Organisms which appear to fit the criteria above but which are not listed here should be assigned to the category of "disabled" host unless a strong case can be made for their inclusion within this category.

Non-pathogenic species

Aspergillus oryzae

Bacillus subtilis

Saccharomyces cerevisiae

Schizosaccharomyces pombe

Rhizobium spp. (inc. Bradyrhizobium)

Derivatives of pathogenic species

5. Especially disabled derivatives of bacterial pathogens such as *E. coli* are those whose growth and survival depends on the addition of nutrients not available in humans or in the environment outside of the culture media and are sensitive to agents present in humans or in the environment. Examples of such mutations are diaminopimelic acid requirement, thymine auxotrophy, streptomycin dependency and deoxycholate sensitivity. This definition currently applies to a limited number of strains of K12 only as follows;

MRC1, MRC7, MRC8, MRC9, X1776 & X1876

Eukaryotic cell & tissue culture systems

6. In addition to the above species, all higher eukaryote cell and tissue culture systems (plant or animal, including mammalian) can be considered as especially disabled hosts provided that the cell line is unable to colonise the worker (i.e. not their own cells) and contains no known adventitious agents which are potentially harmful. The vector used must not be able to infect or transfer DNA to other hosts (see below).

(ii) Disabled or non-colonising hosts

7. This category of host is defined as having biological limitations which mean that it is unlikely to survive in the gut, lung or elsewhere. This description is generally considered to cover laboratory adapted strains (particularly multiply auxotrophic or recombination deficient mutants) as well as other non-pathogenic hosts with negligible demonstrated or suggested capacity to persist in humans and a history of safe use (such as a plant pathogen).

8. Examples include most *E.coli* K12 multiple auxotrophs and other strains and species which are non-pathogenic to humans as listed below:

E. coli **K-12 or B derivatives such as –**

AG1, BW313, CES201, CPLK, C600, DH1, DH5, HB101, INV1, JM83, JM101, JM103, JM105, JM107, JM109, JM110, K808, KW251, LE392, NM554, N99, N4830, NM538, NM5329, P2392, PLK-A, PLK-F, RR1, SCS1, TB1, TG2, XS127, MC106-P3, 71-18, BB4, CSH18, DH20, DH21, NM522, PLK-F', SRB, SURF™ XL1-Blue, Y1088, Y1089, Y1090

Yeasts

Pichia pastori

Other examples

Agrobacterium tumefaciens
Erwinia species
Well characterized derivatives or mutants of *Bacillus brevis, B.sphaericus, B. stearothermophilus and Clostridium* acetobutylicum
Corynebacterium glutamicum
Klebsiella oxytoca M5a1 or KP1.
Lactococcus lactis
Lactobacillus bulgaricus
Lactobacillus helveticus
Lactobacillus plantarum
Salmonella typhimurium, well characterized derivatives such as BRD509, BRD915, BRD917, SL3261 & TA2657
Staphylococcus aureus 8325-4
Staphylococcus camosus
Streptomyces spp,; well characterised strains of species such as *S.coelicolor, S. lividans, S. parvulus* and *S. griseus.*

(i) Other hosts

9. Hosts which do not fall into either of the above categories, i.e. they are not laboratory adapted mutants and/or are capable of infecting or colonising humans or persisting outside of the culture media should be assigned an access factor of 10^{-3} or 1. *E. coli* strain BL21 may fall into this category, as it is not a K12 or B strain derivative and there is little evidence as to the nature of its disablement. On this basis, a value of 10^{-3} is considered appropriate. A rec$^-$ derivitive of the strain is also available, and would warrant a value of 10^{-6}.

10. As a general principle, it is recommended that users working with a wild type strain consider using as alternative, especially disabled or disabled, strains or mutants of the same species. Where a non-disabled strain is used, this should be justified in the risk assessment. In the case of organisms that are biological

agents, there is a requirement to substitute for a less hazardous biological agent where reasonably practicable. However, if there are no suitable alternatives, the following examples may assist in assigning suitable access factors.

11. If a host is known to be pathogenic to humans and appears for example in the List of Biological Agents (Appendix I) then it should automatically be assigned an access factor of 1 and used at a containment level consistent with its hazard group. Examples of this would include most strains of *Salmonella enterica or Staphyloccoccus aureus.*

12. Organisms which are not generally regarded as pathogens but which are capable of colonising the human gut (e.g. *Citrobacter freundii),* respiratory tract (e.g. *Branhamella catarrhalis)* or skin (e.g. *Propionibacterium acnes)* would also generally warrant an access factor of 1.

13. A wild type host which is non-pathogenic or unlikely to colonise humans (for example *Leuconostoc mesenteroides, Pseudomonas putida or Bacillus megaterium* but which is relatively 'robust' and could survive outside of the culture media, would probably (depending on its properties) warrant an access factor of 10^{-3} when used with a non-mobilisable or mobilisation defective vector.

Fungi

14. Most fungi are non-pathogenic and do not colonise humans and many strains or species have a proven and extended history of safe use. However, there are some pathogenic species and certain commonly used species such as *Aspergillus fumigatus* which are allergenic or can cause infections following deep puncture wounds. There is also a large variation in the behaviour of different strains of the same species; laboratory-adapted strains can differ markedly from fresh isolates. For these reasons, it is difficult to reliably assign fungal species to the above categories of disabled or wild type host. The following examples act as a guide to suitable access factors for fungal hosts.

15. A laboratory adapted strain of a non-pathogenic, non-allergenic fungus, for example, *Penicillium crysogenum, Neurospora crassa or Mucor spp.* used with most types of integration vector represents a minimal risk and an access factor of 10^{-9} is appropriate.

16. A laboratory-adapted or Auxotrophic strain of all allergenic or pathogenic fungi, such as *Aspergillus niger or A. nidulans,* used with a vector which does not contain resistance genes to antibiotics used therapeutically against that host, is of low or moderate risk and an access factor of 10^{-6} is generally appropriate.

17. Well characterised wild type fungi with a history of safe use or with biological barriers which will not permit them to colonise or infect humans may be suitable

for an access factor of 10^{-6}. For other wild type, non-pathogenic, fungi without a history of safe use, a value of 10^{-3} would be appropriate.

18. A pathogenic strain or species, for example *Aspergillus fumigatus* or *Sporothrix schenckil* would merit an access factor of 1 and in addition, a containment level consistent with their biological agents Risk Group.

Vector systems

19. The access factor also includes an assessment of the likelihood that a vector can be transferred to another organism. The assignment of an access factor involves classifying the vector as either 'non-mobilisable', 'mobilisation defective' or 'self-mobilisable'. The notes in Table 1 are written based upon *E.coli* plasmids, but the same principles can be used to categorise other vector systems. In order to classify such a vector, information should be available concerning the likely mechanism of transfer (if any) and of any mutations or deleted regions which will reduce transfer.

20. The precise nature of such mutations will depend on the vector, i.e. whether it is based on a plasmid or on a virus. In determining the category of vector, reference should be made to the well-known examples listed below. The vectors in i) and ii) below can generally be considered to be well characterised and poorly mobilisable.

(i) Non-mobilisable vectors

21. These vectors are defective in one or more functions required for transfer to other hosts. For many plasmid vectors, these are loci such as Bom (basis of mobility/bacterial origin of mobility) which is sometimes synonymous with Nic as the site of the origin of transfer (*ori*T); Mob (mobility) which supply a trans-acting peptide which interacts with Bom to promote mobilisation and Tra (transfer) genes which encode the various pili proteins and other DNA processing proteins essential for conjugation.

(ii) Non-mobilisable bactrial plasmid vectors

22. For *E. coli* plasmid vectors they should be Bom$^-$/(Nic$^-$), Mob and Tra. Such vectors include:

pAT153, pACYC 184, pBR327, pBR328, pUC series, pBluescript II, pMTL20, pBS, pGEM, pGEMEX, pGEM Zf, pBS, pUR222, pUCBM, pSP64, pEX series, pCAT series, pT3/T7, pEUK-C1, pEUK-C2, pMAM, pDR720, pR1T2T, pR1T5, pMSG, pSP18, pSP19, pSP6/T3, pSP6/T7, pXT1, pSUB, pEMBL 18, pEMBL19, pSELECT.

Cosmid vectors – *pHC79, pWE15, 16, Super Cos 1, pAA113, pAA113-X, pAA113-M*

23. The following *B. subtilis* vectors can normally be considered to be non-mobilisable:

pUB110, pC194, pS194, pSA2100, pE194, pT127, pUB112, pC221, pC223 & pAB124, pBD series

Yeast vectors:

24. Although yeast do not transfer genes except as part of sexual reproduction, for the purposes of risk assessment, the following vector systems in a standard yeast strain can be considered to be non-mobilisable:

integration vectors (e.g. Ylp vectors)

autonomously replicating vectors using ars sequences (e.g. *Yrp, Ycp, Ylp, YARp, Ypp, Yxp, Yhp or pYAC vectors),*

vectors incorporating portions of the 2um plasmid *(e.g. Yep, Ycp, YARp, YPp, YXp or YHp vectors).*

(When the above yeast/bacteria shuttle vectors are grown in bacterial hosts, the access factor should be based solely on the bacterial components of the system).

Bacteriophage vectors

25. **Lambda vectors:** The criteria for non-mobilisable vectors are also considered to be met by λ vectors which have a restricted host range resulting from any of the following modifications:

Incorporation of one or more suppressible nonsense mutations in essential genes (eg Sam7)

Deletion of the phage attachment site (att) coupled with a defect in the repressor (cl gene) or operator site (eg temperature- sensitive cl857 or cl insertion vectors such as λgt10)

Incorporation of the nin deletion which prevents propagation in the plasmid mode, together with a suppressible nonsense mutation in a essential gene or removal of the phage attachment site, or a defect in the repressor (cl gene) or operator.

26. Examples of non-mobilisable vectors include:
λCharon 3A, λgt10 (and derivatives such as λGEM2, 4 etc)
λgt WES, λEMBL3, 4(and derivatives such as λGEM11, 12),

λgt11 (and derivatives such as λZAP, λDASHII, λFIX).

27. **M13 vectors:** Any M13 vector used in a host containing a tra⁻ F plasmid is considered non-mobilisable.

28. A number of vectors listed as *E.coli* vectors are shuttle vectors intended for transient or stable expression studies in animal or plant cell-lines. Examples such as pMSG, pCH110 and pXT1 are based on eukaryotic viral sequences (mouse mammary tumour virus, SV40 virus and MMLV respectively).

Integrated vectors

29. Vectors which are integrated into the host genome may also be considered non-mobilisable vectors. It is important to consider any mechanisms within the integrated vector which may enable, for example, transposition to other sites or replicons within the host.

(iii) Mobilisation defective vectors

30. There are vectors which are defective in one or more transfer functions and which can only be moblised by other elements which supply the missing functions.

For E. coli, plasmid vectors which are Bom⁺/(Nic⁺) but Tra⁻ and Mob⁻, can be efficiently mobilised if they are co-resident with certain other plasmids. Examples are:
pBR322, pBR325, pET, pACYC177, p15A, pROK-1, pKK233-2, pKK338-1, pBTac1, pBTac-2, pBTrp2, pBTrp56, pKC-30, pKT279, pKT280, pKT287, pFBseries, pNO1523, pSVL, pKSV-10, pGA482, pGA580, pNOS, pHSV-106, RP4 V1.

31. It is especially important to exclude the possibility that a chosen host contains a self-mobilisable plasmid which may provide the defective products *in trans* and allow efficient mobilisation (see below).

Self mobilisable vectors

32. These are vectors which are conjugative or can be mobilised by conjugative plasmids. It also includes bacteriophage vectors which are capable of producing infective phage and infecting other hosts.

Plasmid vectors

33. These are vectors which are either self-transmissable or can be readily mobilised by co-resident
conjugative plasmids

conjugative plasmids i.e. they are $Bom^+/(Nic^+)$, Mob^+ and/or Tra^+.

a) Such plasmids include F, RP4, RSF1010 & ColE1.

b) In determining the presence of self-mobilisable vectors, attention must be paid to the presence of chromosomally-integrated 'helper' plasmids or cloned genes which are intended to mobilise the vector to other cells (for example Ti-based systems in *Agrobacterium).*

34. Certain commonly used *E.coli* strains contain integrated or episomal copies of plasmid F, without the traD36 deletion (or similar) which render it non-conjugative. The use of such hosts with plasmids which can be be mobilised by F may well require an increase in the assigned access factor.

E.coli strains containing Tra^+ F or F' plasmids include:

71-18, BB4, CSH18, DH20, DH21, NM522, PLK-F', SRB, SURE™, XL1-Blue.

Bacteriophage systems

35. Self-mobilisable bacteriophage vectors are those which do not have a limited host range due to mutation, and/or are capable of stable lysogeny. They include wild type λ and M13.

36. The scheme described in Chapter 2 gives a rational basis for determining the appropriate containment for a wide range of hosts and cloned genes. However, there are some additional considerations which affect work with prion protein genes and certain potentially harmful DNA sequences, which may not produce a harmful phenotype in the GMM but might be associated with a harmful or pathogenic phenotype if transferred to another cell type. For the purpose of this guide, harmful DNA sequences are taken to be the following:

- oncogenic sequences
- eukaryotic viral genomic DNA, including potentially infectious DNA derived from RNA viruses (e.g. HIV provirus or cDNA from picomavirus).

B. Information Required for the Certification of New Host/Vector Systems

(i) Characteristics of Donor and Recipient Organisms

1. *Taxonomy, identification, source, culture*

a. Name and designations
b. The degree of relatedness between the donor and recipient organisms and evidence indicating exchange of genetic material by natural means.
c. Characteristic of the organisms which permit identification and the methods used to identify the organisms.
d. Techniques employed in the laboratory and/or environment for detecting the presence of, and for monitoring, numbers of the organism.
e. The sources of the organisms.
f. Information on the recipient organism's reproductive cycle (sexual/asexual).
g. Factors which might limit the reproduction, growth and survival of the recipient organism.

2. *Genetic characteristics of donor and recipient organisms*

a. History of prior genetic manipulation.
b. Characterisation of the recipient and donor genomes.
c. Stability of recipient organisms in terms of relevant genetic traits.

3. *Pathogenic and physiological traits for donor and recipient Organisms*

a. Nature of pathogenecity and virulence, infectivity, or toxicity.
b. Host range.
c. Other potentially significant physiological traits.
d. Stability of these traits.

(ii) Characteristics of the Modified organism

a. Description of the modification.
b. The nature, function and source of the inserted donor nucleic acid, including regulatory or other elements affecting the function of the DNA and of the vector.
c. The method(s) by which the vector with insert(s) has been constructed.
d. Methods for introducing the vector-insert into the recipient organism and the procedure for selection of the modified organism.
e. The structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified organism.

Appendix IV

Risk Assessment

(1) The following matters shall be taken into account in making the assessment for the purposes of regulation –

(a) the identification of any "potentially harmful effects" to humans or the environment, in particular those associated with :

i. the recipient organism,
ii. the inserted (donated) genetic material,
iii the vector,
iv. the donor organism, and
v. the resulting genetically modified organism;

(b) the characteristics of the activity;

(c) the severity of the potentially harmful effects; and

(d) the likelihood of the potentially harmful effects being realised.

(2) In (1) above, "potentially harmful effects" means-

(a) disease to humans including allergenic or toxic effects;

(*) disease to plants or animals

(b) acting as a human, animal or plant disease vector or reservoir;

(c) adverse effects arising from change in behaviour or in physical nature;

(d) adverse effects arising from the inability to treat human, animal or plant disease or offer effective prophylaxis.

(*) ability to survive, establish or disseminate in the environment and cause adverse effects;

(*) adverse effects arising from natural transfer of inserted genetic material to other organisms.

(3) Steps to be included in the risk assessment:

(a) identification of the harmful properties of the recipient and, where appropriate, the donor organism;

(b) identification of any harmful properties associated with the vector or inserted material, including any alteration in the recipient's existing properties;

(c) identification of the level of risk associated with the genetically modified organisms;

(d) selection of appropriate containment and other protective measures on the basis of –

i. the level of risk,
ii. the characteristics of the activity;

(e) adjustment of the level or risk in the light of the matters referred to in the sub-paragraph (d) above; and review and reconsideration of the appropriate containment measures in the light of the completed assessment.

A summary of the risk assessment may be documented in the following format (Form RA).

Documentation of Risk Assessment

Form RA: Summary of the Risk Assessment

(Please complete all boxes, write "not applicable" if not relevant to your activity; * select appropriate category)

Brief Overview of Activity	**Aim/s of project:**
Hazard identification in respect of human health and environmental safety. (Consider host, vector, insert and final GMM.)	**Host Name** **Type*:** Especially disabled/ disabled/ non-colonising / pathogenic/ colonizing/ wild -type / animal virus/plant virus/other **Risk Group*:** RG 1 / 2 / 3 / 4 **Brief description:** **Vector Name:** **Type*:** non-mobilisable / mobilsation defective/ self mobilisable/animal virus /plant virus /other **DNA insert Nature and source** **Type*:** Non-expressible DNA/expressible DNA **Type of Construct*:** Targeting non-expression/ intended limited expression / intended expression / intended to maximize expression/ not applicable **Gene product*:** a biologically active substance, known to be safe/ unlikely to cause harm/ biologically inactive substance of a harmful substance/toxic substance/not applicable

Estimation of the severity or consequence of the harmful effect were it to occur.	**Brief description of assessment:**
Provisional containment level (in particular, taking account of the biological agents hazard group and other classification scheme for pathogens.) This step will often involve considering the containment level necessary to control the risk of the host and making a judgement about whether the modification will result in a GMM which is more hazardous, less hazardous or about the same.	**Type of GMM*** : likely to be similar to host/ likely to be less hazardous/ likely to be more hazardous. **Provisional containment level***: BCL 1/2/3/4. **Brief justification:**

Environment and activity considerations. This includes an estimation of the likelihood that hazards will be realised. Given that the provisional containment level has already been decided, it helps to bear this in mind when deciding how likely a harmful event is. Use these considerations of likelihood to revise the provisional containment so that all risks are controlled to a low of effectively zero. Double check that all hazards are properly controlled by the proposed containment.	**Scale of activity:** **Specific Procedures:** **Control measures for GM animals/plants:** **Additional control measures :** **Other environmental factors considered:**
Assign final activity class. This is done by comparing the containment and control measures identified as necessary to control the risk.	**Final class of activity*** : BCL 1/2/3/4 **Brief Justification:**
Any other relevant information	

Worked examples of Documentation of Risk Assessment :

Eg. 1: Escherichia[1] coli K-12 derivative (e.g. DH5α) expressing human growth hormone.

Brief Overview of Activity	**Aim/s of project:** To clone and express the human growth hormone in an *E.coli* K-12 derivative, DH5∝. The construct will be grown at a pilot plant scale of 15 litres.
Hazard identification in respect of human health and environmental safety. (Consider host, vector, insert and final GMM.)	**Host Name** E.coli K-12 DH5∝ **Type:** Especially disabled/ disabled/ non-colonising / pathogenic/ colonizing/ wild -type /other ☐ **Risk Group:** RG 1 / 2 / 3 / 4 **Brief description:** The host is not considered pathogenic to humans or animals. They are expected to have limited survivability in the environment and often have auxotrophic requirements, which are unlikely to be satisfied outside of laboratory culture. **Vector Name:** pUC18 **Type:** non-mobilisable / mobilsation defective/ self mobilisable/ other ☐ **DNA insert Nature and source** Human growth hormone **Type:** Non-expressible DNA/expressible DNA **Type of Construct :** Targeting non expression/ intended limited expression / intended expression / intended to maximize expression/ not applicable **Gene product:** a biologically active substance, known to be safe/ unlikely to cause harm/ biologically inactive substance of a harmful substance/toxic substance/not applicable

Estimation of the severity or consequence of the harmful effect were it to occur.	**Brief description of assessment**: The human growth hormone is expressed as a fusion protein, which forms insoluble inclusion bodies within bacterial cells. The initially expressed product is thus biologically inactive and it requires treatment by several in vitro laboratory steps to produce active protein. Therefore, while human growth hormone could exert harmful effects if delivered in a biologically active form, for the purposes of the risk assessment the expressed gene product can be considered non-harmful.
Provisional containment level (in particular taking account of the biological agents hazard group and other classification scheme for pathogens.) This step will often involve considering the containment level necessary to control the risk of the host and making a judgement about whether the modification will result in a GMM which is more hazardous, less hazardous or about the same.	**Type of GMM :** likely to be similar to host/less hazardous/ more hazardous. **Provisional containment level:** BCL 1/2/3/4. **Brief justification**: The cloned protein is unlikely to alter the pathogenicity of the cloning host, and is likely to reduce it's survivability/ fitness. The host is biological agents Risk Group 1, for which BCL 1 is appropriate.
Environment and activity considerations. This includes an estimation of the likelihood that hazards will be realised. Given that the provisional containment level has already been decided, it helps to bear this in mind when deciding how likely a harmful event is.	**Scale of activity:** 10 litres **Specific Procedures**: The genetically modified micro-organism (GMM) is being grown at large scale (10 litres), under mono-sepetic conditions. It is being grown in a closed stainless steel fermenter, and will be harvested by centrifugation. The paste will be passed through a cell disrupter, and the insoluble inclusion bodies harvested. The centrate, containing cell debris and a low titre of viable cells, will be heat inactivated and discharged. **Control measures for GM animals/plants:** Not applicable.

Use these considerations of likelihood to revise the provisional containment so that all risks are controlled to a low of effectively zero. Double check that all hazards are properly controlled by the proposed containment.	**Additional control measures** : Although the fermenter will be completely contained, with appropriate seals being used, and with off gases being filtered, these measures are primarily to prevent contamination, and are in excess of what would be required for the purposes of protection of human health or the environment. **Other environmental factors considered** : In the event of spillage from the fermenter, the area can be effectivel disinfected. The fermenter is housed within a process building, and the wider environment is unlikely to become contaminated
Assign final activity class. This is done by comparing the containment and control measures identified as necessary to control the risk.	**Final class of activity :** BCL 1/2/3/4 **Brief Justification**: For laboratory operations, a standard BCL 1 facility, and the use of good microbiological practice will be sufficient to limit contact with humans and the environment. For the large scale operations, the process equipment used will be sufficient to limit contact. The organism is unlikely to cause harm to either workers or the environment, so filtration of the off gasses, and the use of a closed system is not required as a safety measure. None of the measures in BCL 2 is required for safety reasons, even though they will be used for process reasons.
Any other relevant information	

Eg.2 Genetic manipulation of foot and mouth disease virus

Brief Overview of Activity	**Aim/s of project:** To introduce mutations into foot and mouth disease virus (FMDV) to study the function of targeted genes.
Hazard identification in respect of human health and environmental safety. (Consider host, vector, insert and final GMM.)	**Host Name** FMDV **Type:** Especially disabled/ disabled/ non-colonising / pathogenic/ colonizing/ wild -type / animal virus/plant virus/other ☐ **Risk Group:** RG 1 / 2 / 3 / 4 **Brief description:** Live FMDV will be recovered from ful length cDNA clones of the RNA genome. Critical residues wil be mutated, and the effect on the recovered virus will b analysed. **Vector Name:** FMDV **Type:** non-mobilisable / mobilsation defective/ self mobilisable/animal virus /plant virus /other ☐ **DNA insert Nature and source** Not applicable **Type:** Non-expressible DNA/expressible DNA **Type of Construct** : Targeting non expression/ intended limited expression / intended expression / intended to maximize expression/ not applicable **Gene product:** a biologically active substance, known to be safe/ unlikely to cause harm/ biologically inactive substance of a harmful substance/toxic substance/not applicable

Estimation of the severity or consequence of the harmful effect were it to occur.	**Brief description of assessment:** The FMDV can be considered to be both the host and the vector. FMDV is an Aphthovirus, and is predominantly considered to be an animal pathogen. It is classified as requiring BCL 4. It infects most species of domestic and wild cloven hooved animals as well as rodents. It is highly infectious through ingestion and the airborne aerosol route. Infection in man has been reported, and some cases of laboratory acquired infection recorded. The modifications being made to the virus involve mutating, or introducing deletions into genes to study function *in vitro*. At a later stage these constructs may be introduced into animals, however, a separate notification will be made for such studies. The modifications are expected to have an adverse effect on the virus, reducing it's fitness. However, as the studies are aimed at elucidating the function of genes, it cannot be certain that all modifications will lead to a less harmful virus. The main concern with FMDV is environmental. It causes serious disease in animals, and severe economic losses in terms of meat and milk production. Human exposure can lead to symptoms, but not serious disease.
Provisional containment level (in particular taking account of the biological agents hazard group and other classification scheme for pathogens.) This step will often involve considering the containment level necessary to control the risk of the host and making a judgement about whether the modification will result in a GMM which is more hazardous, less hazardous or about the same.	**Brief justification**: FMDV is a RG 4 pathogen. The containment requirements are aimed primarily at protecting the environment through preventing escape of pathogens from the laboratory, rather than protecting the health of workers. However, all work with live virus will be carried out in a safety cabinet, to reduce worker exposure. Air from the laboratory would be extracted via two HEPA filters in series, and the supply air filtered through a single HEPA filter. Entrance to and exit from the facility would be via an airlock which will be ventilated through an air exhaust system. The laboratory will be maintained at negative pressure; a full change of clothes will be done, all waste will be autoclaved through a double ended autoclave; the laboratory will be sealable for fumigation. It is not thought that the modifications will increase the pathogenicity of the virus, so additional measures above those for the wild type virus are not required.

Environment and activity considerations. This includes an estimation of the likelihood that hazards will be realised. Given that the provisional containment level has already been decided, it helps to bear this in mind when deciding how likely a harmful event is. Use these considerations of likelihood to revise the provisional containment so that all risks are controlled to a low of effectively zero. Double check that all hazards are properly controlled by the proposed containment.	**Scale of activity:** Not applicable **Specific Procedures:** If all the precautions listed in BCL 4 are implemented, it is unlikely that the environment surrounding the facility will be exposed to the virus, and the risk can be considered to be effectively zero. **Control measures for GM animals/plants:** Not applicable. **Additional control measures :** There is a possibility that workers could transmit the virus from the laboratory, and it could spread into the wider environment. The use of gloves, safety cabinets, showering out, as well as local rules on contact with animals should mitigate against this happening. **Other environmental factors considered :** The virus is primarily an animal pathogen, and protection of the environment is the primary concern. Full implementation of BCL 4 should protect the environment. As the virus is not considered to be a serious human pathogen, so a Class III cabinet is not considered necessary for handling live virus.
Assign final activity class. This is done by comparing the containment and control measures identified as necessary to control the risk.	**Final class of activity :** BCL 1/2/3/4 **Brief Justification:** A number of the key containment measures required for handling the virus are consistent with those in BCL 4 – the input and extract air will be filtered, complete change of clothing will be done, and the double ended autoclave activated. The operation should therefore be notified as a BCL 4 activity. Full level 4 (such as the use of a Class III cabinet) may not be required, as the virus is not a serious human pathogen.
Any other relevant information	

For Official Use only
Ref. No:
Date of receipt:
Date acknowledged:
Date approved (if applicable):

APPENDIX V

FORM N: Notification of individual activities involving recombinant DNA (rDNA) molecules

Instructions for use:

- Before completing this form, please read the "Guidelines for the safe use of recombinant DNA technology in the laboratory" published by the National Science Foundation, Sri Lanka
- This form should be completed by all investigators intending to initiate rDNA Category II and Category III work. Please return the completed form (Sections 1-3) to the Institutional Biosafety Committee. (The Institutional Address is given at the end of the form.) Two copies of the completed form should be submitted, a copy of which will be returned to the applicant after completing section 4 of the form by the IBSC.
- Those intending to conduct rDNACategory III work need written consent from the Institutional Biosafety Committee prior to commencement of such rDNA work.

1. Details of work site and persons responsible for the work to be carried out (if the project requires rDNA work to be conducted in more than one institute, the relevant institutes must be notified by the project counterparts of each institute).

(i)	Principal Investigator/ Project Counterpart of Institute	
	Department & Address	
	Position	
	Telephone/Fax Numbers & E-mail address	
(ii)	Co-investigator(s)	
(iii)	List all personnel associated with activity (including self)	Qualifications (Degree) &Training /Experience (Systems and the Number of Years)
	1.	
	2.	
	3.	
	4.	
	5.	
	6.	
	7.	

2. Details of rDNA activity

(i)	Activity to be undertaken including expected results:	
(ii)	Funding Source (if applicable)	
(iii)	Are the activity procedures identical to a previously approved project? YES/ NO If YES, give the reference number and the date of approval :	
(iv)	Is the activity an amendment to a previously approved project? YES / NO If YES, give the reference number and the date of approval:	
(v)	Is it a new activity? YES / NO	
(vi)	List Host System(s) to be used:	
(vii)	List Vector(s) to be used:	
(viii)	List Nature and Source(s) of DNA to be inserted:	
(ix)	Will a deliberate attempt be made to obtain expression of a gene product? YES/NO If YES, list what protein will be produced:	
(x)	Select Containment Level required for the activity Do you have proper facilities for the safe conduct of the above work? List available facilities and waste treatment procedures: If the answer to the above is NO, what action will be taken to establish procedures? Give the expected date of achievement:	BCL I /BCL 2/ BCL 3/ BCL 4 ; Plant A/B ; Animal A /B / C YES / NO .
(xi)	Select Category of rDNA activity as specified by the Guidelines (NSF) - rDNA II / rDNA III	
(xii)	Please tick to confirm that you have enclosed a summary of risk assessment YES	

3. Declaration by the Principal Investigator:

I have read the "Guidelines for the safe use of recombinant DNA technology in the laboratory" published by the National Science Foundation, Sri Lanka, and I am familiar with the provisions of the current Guidelines which pertain to this project. I acknowledge my responsibility to comply with the requirements of the Guidelines for the safe conduct of all rDNA activities described herein. I agree to accept responsibility for the training of all laboratory personnel involved in the project. The information submitted by me in this document is accurate and complete to the best of my knowledge. If the rDNA activity described herein is classified under Category III, I will not begin this work until it is approved by the Institutional Biosafety Committee.

Signature of the Principal Investigator & Date:

4. This section is to be filled only by the IBSC. One copy of Form N must be returned to the Principal Investigator after completion of the following sections (where applicable) by the IBSC.

(i) Observations made by the Institutional Bio- Safety Committee:

(ii) Date of acknowledgement of notification :
(iii) Signature of the Chairperson of IBSC :

(iv) This section is to be filled for rDNA Category III activities only:

Action taken by the Institutional Biosafety Committee:

(a) Activity is approved as submitted. ☐
(b) Activity is approved with modifications ☐
(c) Permission is not granted for the activity ☐

The space provided could be used to write the modifications needed (b) or the reasons for not granting approval for the proposed work (c):

Signature of Chairperson of IBSC and Date:

For approved activities only :

I agree to act as Biosafety Officer in connection with the proposed work in this submission.

Signature of Biosafety Officer and Date:

APPENDIX VI

Containment Measures for Activities in Laboratories

	Containment Measures	Biosafety Containment Level			
		1	2	3	4
1	Laboratory suite: isolation **(Note 1)**	not required	not required	not required	not required
2	Laboratory: sealable for fumigation	Not required	Not required	Required	Required
	Equipment				
3	Surfaces impervious to water and resistant to acids, alkalis, solvents, disinfectants, decontamination agents and easy to clean	Required for bench	Required for bench	Required for bench and floor	Required for bench, floor, ceiling and walls
4	Entry to lab via airlock **(Note 2)**	Not required	Not required	Required where, and to extend the risk assessment shows it is required	Required
5	Negative pressure relative to the pressure of the immediate surroundings	Not required	Required where, and to extent the risk assessment shown it is required	Required	Required
6	Extract and input air from the laboratory should be HEPA filtered	Not required	Not required	HEPA filters required for extract air	HEPA filters required for input and extract air **(Note 3)**
7	Micro biological safety cabinet/enclosure	Not required	Required where, and to extent the risk assessment shows it is required	Required, and all procedures with ineffective materials required to be contained within a cabinet/enclosure	Class III cabinet required
8	Autoclave	Required on site	Required in the building	Required in the laboratory suite **(Note 4)**	Double ended autoclave required in laboratory
	System of work				
9	Access restricted to authorized personnel only	Not required	Required	Required	Required via airlock key procedure
10	Specific measures to control aerosol dissemination	Not required	Required so as to minimize	Required so as to prevent	Required so as to prevent
11	Shower	Not required	Not required	Required where, and to extent the risk assessment shows it is required	Required
12	Protective clothing	Suitable	Suitable	Suitable protective	Complete

		protective clothing required	protective clothing required	clothing required; footwear required where, and to extent the risk assessment shows it is required	change of clothing and footwear required before entry and exit
13	Gloves	Not required	Required where, and to extent the risk assessment shows it is required	Required	Required
14	Effective control of disease vectors (eg for rodents and insects) which could disseminate GMMs	Required where, and to extent the risk assessment shows it is required	Required	Required	Required
15	Specified disinfection procedures in place	Required where and to extent the risk assessment shows it is required	Required	Required	Required
	Waste				
16	Inactivation of GMMs in effluent from handwashing sinks and showers and similar effluents	Not required	Not required	Required where, and to extent the risk assessment shows it is required	Required
17	Inactivation of GMMs in contaminated material and waste	Required by validated means	Required by validated means	Required by validated means	Required by validated means
	Other measures				
18	Laboratory to contain its own equipment	Not required	Not required	Required, so far as is reasonably practicable	Required
19	An observation window or alternative is to be present so that occupants can be seen	Required where, and to extent the risk assessment shows it is required	Required where, and to extent the risk assessment shows it is required	Required	Required
20	Safe storage of GMMs	Required where, and to extent the risk assessment shows it is required	Required	Required	Secure storage required

21	Written records of staff training	Not required	Required where, and to extent the risk assessment shows it is required	Required	Required

NOTES

1. In the table above, "isolation" means, in relation to a laboratory, separation of the laboratory, separation of the laboratory from other areas in the same building, or being in a separate building.

2. Entry must be through an airlock which is a chamber isolated from the laboratory. The clean side of the airlock must be separated from the restricted side by changing or showering facilities and preferably by interlocking doors.

3. Where viruses are not retained by the HEPA filters, extra requirements will be necessary for extract air.

4. Where the autoclave is outside the laboratory in which the contained use activity is being undertaken, but within the laboratory suite, there shall be validated procedures for the safe transfer of material into that autoclave, which provide a level of protection equivalent to that which would be achieved by having an autoclave in that laboratory.

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