A PRACTICAL MANUAL FOR CONTAINMENT

A Guide to a Safe Conduct of Laboratory and Greenhouse Research with Genetically Modified Organisms (GMOs)

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CONTENTS

ABBREVIATIONS AND ACRONYMS

BL	Biosafety Level
CFT	Contained research
CPB	Cartagena Protocol on Biosafety
DNA	Deoxyribonucleic Acid
GEP	Genetically Engineered Plant
GMO	Genetically Modified Organism
HEPA	High Efficiency Particulate Air
HV	Host-Vector
IBC	Institutional Biosafety Committee
MARI	Mikocheni Agricultural Research Institute
NBFP	National Biosafety Focal Point
RL	Risk Level
SOP	Standard Operating Procedures (SOPs).
UNEP	United Nations Environment Program
VPO	Vice President's Office.
WHO	World Health Organisation

DEFINITION OF TERMS

Accidental Release: Any unauthorized release of regulated GE plants and plant products in the environment; the human food and/or livestock feed chains.

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

Biosafety: Avoidance of risk to the protection of the environment and to human and animal health, as a result of the use for research and commerce of GMOs, by instituting legal, administrative and policy instruments

Biotechnology: Any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use.

Construct: A segment of DNA to be transferred into a cell or tissue and type using a specific genetic modification

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

Containment: the use of physical, chemical, operational or biological controls (or a combination thereof) within a contained facility (*e.g.*, a laboratory or animal housing facility) to restrict contact of microorganisms or organisms other than microorganisms with humans and the environment. The containment concept also applies to transport and disposal.

Genetically Engineered (GE) Plants and Plant Products: Are plants and plant products developed through genetic medication of organisms by recombinant DNA techniques.

Genetically Modified Organism: Any organism which has been altered or produced through genetic engineering, or any product which contains such an organism, or any other organism or product altered or produced through genetic engineering. For the purpose of this guideline the terms genetically engineered plants, genetically modified plants and transgenic plants should be used interchangeably.

Incident: Any occurrence that causes, or threatens to cause, a breach of confinement of GM plant material

Living organism: Any biological entity capable of transferring or replicating genetic material, including sterile organisms, viruses and viroids.

1. INTRODUCTION

Biotechnology offers tremendous potential for improving agriculture, industry, health care (human and animal) and environment. It can provide scientists with new ways to develop higher-yielding and more nutritious crop varieties, to improve resistance to disease, or to reduce the need for inputs of fertilizers and other expensive agricultural chemicals. Although most biotechnology products are considered safe, genetically modified organisms (GMOs) are subject to special rules intended to ensure that they are used in a way that does not pose an unacceptable risk to human and animal health or the environment. During genetic modification for desired traits, handling of microorganisms or rDNA molecules may result contamination of the environment or personnel. Laboratory workers can minimize the risks associated with working with these agents through the application of appropriate biosafety and containment principles and practices.

Biosafety information about safe handling of genetically Modified (GM) material in laboratories and greenhouses is relatively sparse. Besides, there is no single source that provides practical guidance on managing contained laboratories, safe handling of greenhouses and requirements for building contained facilities to make them suitable for handling genetically modified plants, microorganism and associated organisms. This manual is intended to guide government, industry, university, research and other public and private laboratories in their development of biosafety policies and programs. It will also act as a reference to help clarify what levels of containment is needed and what measures are sufficient to achieve the various biosafety levels.

Guidelines and procedure alone cannot guarantee safety. The safe conduct of contained trials can only be accomplished through the combination of a robust regulatory framework, science-based risk mitigation measures, vigilant inspection staff, and trained laboratory and screenhouse personnel dedicated to abiding by the terms and conditions of containment. It is the responsibility of everyone, including managers and laboratory workers, to use the information available in these Guidelines and to perform their work with diligence, responsibility and in a safe and secure manner.

In recognition of the Guidelines' impact on key stakeholders, a consultation was done widely in order to offer stakeholders an opportunity to state their opinions and comment on the implications of the procedures and containment measures recommended. All comments and feedback that stakeholders provided were reviewed and incorporated in the manual where possible.

It is recommended that the GM risk assessment and the applied containment level be reviewed on a periodic basis, especially if the containment measures employed are no longer suitable or the risk class of the operation has changed. This may also be the case when new scientific knowledge suggests that the initial risk assessment maybe no longer correct.

1.1. Scope

This Handbook provides instructions guidelines and standard Operating Procedures (SOPs) for all aspects of biosafety for contained field trials involving genetically modified organisms (GMOs) in Tanzania. The guide applies to laboratory and green houses and provides detailed description of screenhouse construction, upgrades and other contained plant growth facilities, such as growth chambers, biosafety cabinets, incubators, and tissue culture tables or rooms,

which are often are an integral part of the process leading to the preparation of GMO materials for greenhouse studies or field tests.

This Guide was written so that anyone who works in contained facilities (laboratory and greenhouses) that houses genetically modified materials will be better informed about the purpose of containment, the variety of methods used to achieve it, and the facilities and practices that satisfy the requirements of established guidelines and regulations.

The procedures provided here are for the use of all laboratory managers, technical and research personnel, agents of the Authorized Party, members of Institutional Biosafety Committees (IBC) and National Biosafety Committees (NBC), government officials engaged in planning, conducting or overseeing contained research of GMOs in Tanzania. In addition, designers working on construction of these type of facilities and others who work in and around such facilities, including cleaners, maintenance personnel, and adjacent residents, will benefit from a basic understanding of the purpose of containment. Such understanding will help ensure that GMOs are handled in an environmentally responsible manner.

This manual is intended as a guide and should not be a considered an authoritative source. It was prepared with view to ensuring complementarities and mutual supportiveness with the national policies and legislation. Readers are encouraged to seek additional guidance from Ministry responsible for biosasety, institutional authorities and other regulatory authorities should questions arise.

1.2. Objectives

The Guidelines seek to facilitate the development and enhancement of national capacities to ensure that GM research conducted in laboratories and greenhouses. This would be achieved through the following objectives:

- To provide a simple and convenient reference on appropriate biosafety and containment levels for GMO research conducted in laboratory and greenhouse
- To clarify containment measure needed and suggest measures that are sufficient to achieve the various biosafety levels.
- To ensure the relevant authorities, institutions and other users of the contained facility are informed and have access to the information of the safety, risk assessment and contained measures required

2. General requirements for containment/contained use

2.1. Background to containment

In general, all work that involves recombinant DNA molecule should be performed under containment (References including TZ). Containment, or contained use, refers to measures and protocols applied to reduce contact of GMOs or pathogens with the external environment in order to limit their possible negative consequences on human health and the environment (FAO, 2001). Containment measures have to be adjusted to the highest level of risk associated with the experiment, especially when the risk category of the material being worked with is not certain. The risk associated with each GMO should be assessed on a case-by-case basis; accordingly, GMO s are classified into four different risk groups in relation to the risks they pose (Table 1).

Containment can be achieved by a combination of physical containment structures and safe work procedures (also referred to as good laboratory practices). As an additional feature, biological containment can be included, i.e. "built-in" features of the organism being worked with that prevent its spread, survival or reproduction in the external environment. Appropriate containment measures should be applied at each stage of an experiment involving GMO s to avoid release into the external environment and prevent harmful events. This overall objective of a containment system is always the same, however the actual measures that are required can differ, depending on the organisms being worked with (micro-organisms, plants, animals), the scale of the application (large-scale versus small-scale), the research setting (laboratory, greenhouse) and of course the risk classification of the GMO s.

The basic structure of a containment facility must meet minimum standards appropriate for the category of risk of the work being conducted. Establishment of the basic minimum structure, adherence to general safety requirements and adoption of good laboratory practices specified for a certain risk group enable any work identified as part of that risk group to be performed within that facility. Therefore, the first step in any operation dealing with GMO s is to classify the GMO and the associated work procedures into one of the four risk groups. Subsequently, one can easily identify the required minimum facility features and good laboratory practices associated with that risk group, and check if the facility that is designated to be used and the standard operating procedures (SOP) for the personnel that are in place comply with these requirements.

2.2. Risk assessment and classification

1.1.1 Risk classification

The most common risk classification system is based on four different risk groups, associated with four different biosafety levels (WHO, 2004; NIH, 2009. Risk groups 1 to 4 represent increasing risk to human health and the environment, similarly biosafety levels 1 to 4 represent increasing strength in the containment measures required to prevent dissemination and spread of the organisms being worked with. To establish the classification of a GMO , a comprehensive risk assessment should be performed on a case-by-case basis. An initial assessment can be made by classifying an organism according to the following criteria (Table 1).

Table 1. Classification of infective microorganisms, plants and animals by risk group

	Risk group	Description
1	Risk Group 1 *(low individual and community risk)*	• A microorganism that is unlikely to cause human or animal disease • Plants that are not noxious weeds or cannot cross with weeds. • Stock animals after quarantine and animals that are deliberately inoculated with microorganisms belonging to Risk Group 1
2	Risk Group 2 *(moderate individual risk, low community risk)*	• A microorganism that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited • Plants that are noxious weeds or can interbreed with weeds but would have a negligible impact or could be readily managed. Plants containing genomes of non- exotic infectious agents. • Animals that are deliberately inoculated with microorganisms belonging to Risk Group 2.
3	Risk Group 3 *(high individual risk, low community risk)*	• A microorganism that usually causes serious/lethal human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are/may be available. • Plants infected with exotic infectious agents capable of causing serious environmental harm. • Plants containing vertebrate toxin. • Animals that are deliberately inoculated with microorganisms belonging to Risk Group 3.
4	Risk Group 4 *(high individual risk, high community risk)*	• A microorganism that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available. • Plants involved in experiments on readily transmissible exotic infectious a gents that are potentially serious pathogens to major crops, and are performed in the presence of their arthropod vectors • Animals that are deliberately inoculated with microorganisms belonging to Risk Group 4

Source: Developed from: NIH, 2009; WHO, 2004 and Traynor *et al*., 2001

Risk assessment

A comprehensive **risk assessment** should take a detailed look at the organism and the type of genetic manipulation that it is subjected to; factors to be taken into consideration include virulence, pathogenicity, infectious dose, environmental stability, route of spread, communicability, laboratory operations, quantity being worked with, availability of vaccine or treatment and gene product effects such as toxicity, physiological activity, and allergenicity (NIH, 2009). Such considerations should result in a classification of the organism/project into one of the four risk groups, which also defines the containment level (Table 2) that applies (usually the containment level is the same as the risk group).

Table 2: Relation of risk groups for microorganisms, plants and animals to biosafety levels, practices and equipment*

Risk Group	Biosafety Level	Laboratory practice and equipment
RG1	BL1	Work is generally conducted on bench tops using standard good laboratory practices (GLPs). The laboratory is not necessarily separated from the general traffic patterns in the building. Special containment equipment or special facility design is neither required nor generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in plant transformation.
RG2	BL2	**In addition to measures in BS-1:**Laboratories are clearly labeled with biohazard signs; laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists; access to the laboratory is limited when work is being conducted; extreme precautions are taken with contaminated sharp items; certain procedures are conducted in biological safety cabinets or other physical containment equipment; directional airflow to prevent flow of air into other laboratories; proper decontamination of waste before disposal.
RG3	BL3	**In addition to measures in BS-2:** laboratory personnel have specific training in handling pathogenic and potentially lethal agents, and are supervised by competent scientists who are experienced in working with these agents; all procedures involving the manipulation of infectious materials are conducted within biological safety cabinets or other physical containment device, or by personnel wearing appropriate personnel protective clothing and equipment; laboratory fitted with a double entry door; laboratory has special engineering and design features; a written contingency plan for handling laboratory accidents involving potential hazardous agents is available.
RG4	BL4	**In addition to measures in BS-3:** laboratory staff have specific and thorough training in handling extremely hazardous infectious agents and they understand the primary and secondary containment functions of the standard and special practices, the containment equipment, and the laboratory design characteristics; laboratory staff are supervised by

		competent scientists who are trained and experienced in working with these agents; access to the laboratory is strictly controlled by the individual(s) in charge of the laboratory; the facility is either in a separate building or in a controlled area within a building, which is completely isolated from all areas of the building; a specific facility operations manual is prepared or adopted for working in the laboratory; the laboratory has special engineering and design features to prevent agents from being disseminated into the environment; within the laboratory appropriate specialized safety cabinets are fitted.

Source: Prepared from: ABNE,,,,,

3. Containment of Genetically Modified Organisms

To ensure biosafety in transformation experiments, safety measures are instituted in the laboratory or greenhouse depending on the containment level required for the kind of agents being handled. Containment is achieved through a combination of physical containment measures and safe working practices. The containment levels used to categorize laboratories are designated in ascending order, by degree of protection provided to personnel, the environment, and the community. Below are brief descriptions of laboratory biosafety containment levels 1-4. Note that virtually all plant transformation experiments are performed at biosafety level 1 or 2. Levels 3 and 4 apply to laboratories handling human or animal pathogenic agents.

3.1. Containment of Genetically Plants (GMP)

In this Manual, plants shall be defined in a broad sense and include higher (vascular) plants, including their reproductive organs such as spores, pollen, seeds, tubers, bulbs, rhizomes, as well as mosses, ferns, algae and aquatic species. The manual will provide guidelines for research conducted in (i) laboratories (Section 3.1.2) and (ii) specialized greenhouses (Section 3.1.3)

3.1.1 Risk assessment and classification

Risk assessment

The general principle for a GM plant risk assessment is identical to other GMO operations; however, for plants the potential adverse effects on the environment are in many cases the primary source of concern, which needs to be taken into account during the risk assessment. The comprehensive GM plant risk assessment should consist of the following steps (Health and Safety Executive, 2007):

- identification of potential hazards and evaluation of the likelihood that these hazards are realized;
- evaluation of the consequences should these hazards be realized;
- assessment of the risk, i.e. the likelihood of hazard realization and estimated consequences; and

- assignment of a risk group and assignment of containment measures appropriate for that risk group.

Risk classification

The ultimate goal of the risk assessment procedure is the assignment of the specific activity with a GM plant to one of four risk classes, and the concomitant definition of containment measures required to control and minimize the risks associated with that risk class.

The four risk classes and associated containment measures, also known as biosafety levels for plants 1 to 4 (BL1-P to BL4-P) have been defined by NIH (NIH, 2009, FAO 2011); brief descriptions of each level are discussed below. Biosafety levels constitute a combination of facility features and equipment, work practices and procedures, and administrative measures required to maintain a specified level of containment, with the aim of preventing contact between the material being worked with and the outside environment to the appropriate extent. A detailed table summarizing the exact containment measures associated with each biosafety level for plants is provided in Annex 2.

Biosafety Level 1 (BL1)

The BL1 facility provides for a low level of containment and is suitable for work involving well characterized agents not known to consistently cause disease in healthy adult humans, and of minimal potential harm to laboratory personnel and the environment. This level applies to the basic laboratory and facilities that handle organisms that cannot spread rapidly and are not known to have any negative effects and if accidentally released, would not pose any environmental risk (e.g. Escherichia coli and Agrobacterium spp). The BLI also applies to experiments involving GM plants in which there is no evidence that the modified organism would be able to survive and spread in the environment if accidentally released. It also applies to plants genetically modified with genes from the same species for example an experiment designed to study transgenic potato plants containing cloned genes for insect resistance obtained from primitive potato cultivars. BL1 is also suitable for the maintenance of most stock animals after quarantine and for animals that are deliberately inoculated with microorganism belonging Risk Group 1.

BL1 requires no special design features beyond those suitable for a well designed and functional laboratory and facilities. Biological safety cabinets (BSCs) are not required. Work may be done on an open bench top, and containment is achieved through the use of good laboratory practices.

Biosafety Level 2 (BL2)

This level applies to contained laboratories and facilities that handle organisms that could be viable in the surrounding environment but would have a negligible impact or could be readily managed. The primary exposure hazards associated with organisms requiring BL2 are through the ingestion, inoculation and mucous membrane route. Agents requiring BL2 facilities are not generally transmitted by airborne routes, but care must be taken to avoid the generation of aerosols (aerosols can settle on bench tops and become an ingestion hazard through contamination of the hands) or splashes. BL2 is required for transgenic plants that may exhibit weedy characteristic or that may be capable of interbreeding with weeds. For example,

greenhouse tests of transgenic sunflower containing wheat genes intended to confer resistance to the fungus Sclerotinia because sunflower is capable both of hybridizing with wild relatives, and becoming established as a volunteer weed. BL2 containment is assigned to transgenic experiments that use the entire genome of non-exotic infectious agents. This level of containment is also appropriate for experiments of animals that are deliberately inoculated with microorganisms belonging to Risk Group 2.

Primary containment devices such as BSCs and centrifuges with sealed rotors or safety cups are to be used as well as appropriate personal protective equipment (i.e., gloves, laboratory coats, protective eyewear). Environmental contamination must be minimized by the use of hand-washing sinks and decontamination facilities (autoclaves).

Biosafety Level 3 (BL3)

This is designed to prevent the accidental release of transgenic plants, animals or microorganisms that have a recognized potential for significant detrimental impact on the environment. These organisms may be transmitted by the airborne route, often have a low infectious dose to produce effects and can cause serious or life-threatening disease. BL3 applies to plant research that involves exotic infectious agents capable of causing serious environmental harm or transgenic plants containing genes from an exotic infectious agent in which a complete functional genome of the infectious agent could possibly be reconstituted. Experiments using transgenic plants or organisms that contain genes coding for vertebrate toxins should be conducted in BL3. For example inoculation of transgenic groundnut plants that contains fungal resistance genes with Aspergillus flavus, the organism responsible for producing the potent vertebrate mycotoxin, aflatoxin. BL3 is also suitable for works with animals that are deliberately inoculated with agents belonging to Risk Group 3, or when otherwise indicated by a risk assessment.

BL3 emphasizes additional primary and secondary barriers to minimize the release of infectious organisms, plants and animals into the surrounding environment. Additional features to prevent transmission of BL3 organisms are appropriate respiratory protection, HEPA filtration of exhausted laboratory air and strictly controlled laboratory access.

Biosafety Level 4 (BL4)

This is the maximum containment available. This level is recommended for experiments involving certain exotic, readily transmissible infectious agents that are potentially serious pathogens and that are performed in the presence of their arthropod vector. For example, an experiment to test the efficacy of the African cassava mosaic virus coat protein to protect cassava plants against infection by that virus would necessitate use of a whitefly vector, Bemesia tabaci, in challenge inoculations. This devastating virus is not found in Tanzania, however the vector is present, therefore, and such an experiment poses a significant risk should the virus or vector escape the containment facility. BL4 is also suitable for organisms that have the potential for aerosol transmission, often have a low infectious dose and produce very serious and often fatal disease; there is generally no treatment or vaccine available. Also included in BL4 are certain biopharming experiments in which bioactive compounds (eg vaccines) are produced in GM plants.

Housing areas for animals infected with Risk Group 4 agents must also maintain the criteria for maximum containment described for BL4. This level of containment represents an isolated unit, functionally and, when necessary, structurally independent of other areas. BL4 emphasizes maximum containment by complete sealing of the facility perimeter with confirmation by pressure decay testing; isolation of the researcher from the pathogen by his or her containment in a positive pressure suit or containment of the pathogen in a Class III BSC line; and decontamination of air and other effluents produced in the facility.

3.1.2 Containment measure for GMP in laboratory

The detailed procedures and parameters for safe handling of GM materials in laboratory setting are given in Annex 1 and Annex 2. Other contained facilities such as growth chambers, biosafety cabinets, incubators, and tissue culture tables and rooms, which are an integral part of the process leading to the preparation of GMO materials for green house studies or field tests are described in Section 4.2.

3.1.3 Containment measure for GMP in greenhouses

Greenhouses are specialized structures with a transparent or translucent covering enabling the growth of plants inside a controlled environment. Such structures, and the concomitant work procedures, differ significantly from typical laboratory settings and require special considerations regarding containment.

The primary objective of plant containment is protection to reduce risks to environment, human and animal health. In order to achieve this goal it is recommended to carefully consider all factors that might interfere with containment, including characteristics and behaviour of the organisms being worked with, organism interactions, conduct of experiments, facility (greenhouse) design and limitations, escape routes, and social (personnel-related) factors. A large variety of transport mechanisms for organisms – ranging from micro-organisms to plants – into and out of a containment facility exists, and likewise many opportunities for breaches of containment. These routes include air, water and soil, as well as via personnel (clothing, shoes, etc.), equipment, waste, or via small animal intruders.

Containment measures specifically for greenhouses directed against those factors are briefly described below, while the exact requirements for each plant biosafety level can be found in Annex 3.

- All personnel working in the facility should be familiar with the containment requirements and the work procedures to be followed; SOPs and a reference manual should be established and followed. Problems should be noted and investigated as soon as they become apparent. Routine access should be restricted.
- Care should be taken that dissemination of organisms through clothing, shoes etc. is prevented. Wearing laboratory coats and gloves is recommended even at lower biosafety levels where such measures are not compulsory.
- Physical containment is provided by the facility itself and by equipment employed within that facility; correct handling of the facility and the equipment is required to maintain containment.

- Signs advising of restricted experiments in progress, limited access, potential hazards and contact details of responsible persons should be in place.
- The capability of a greenhouse to isolate organisms from the surrounding environment, as well as to limit entrance of undesired organisms, is strongly affected by the type of glazing, sealing, screening, airflow system, air filtration and air pressure employed.
- Layering of containment measures, i.e. combining several physical measures or combining physical with biological containment measures, can significantly enhance containment (Table 3).
- Special care should be taken when work involves plant-associated micro-organisms, whether or not they are genetically modified themselves. In such cases, the containment measures for micro-organisms should additionally be consulted.
- Storage of material (plant parts, cell culture, seeds) should preferably be performed in lockable repositories.
- Specific requirements exist for safe transfer of material into or out of the facility (use of closed containers, possibly in two layers).
- Prior to disposal, biological material (including soil) must be rendered inactive by validated means (autoclaving recommended).
- Periodic cleaning, as well as disinfection or decontamination of all surfaces or the entire facility should be performed, by means that are efficient for the target organism.
- A pest and undesired organism control programme should be in place; traps or bioindicators can be employed to monitor spread of pollen, insects or viruses etc.
- Alarm systems should be operational to indicate system failures due to technical, human or weather-caused errors and malfunctions.
- Records of experiments should be kept; greenhouses should be inspected periodically.
- Security measures to limit access of unauthorized persons should be in place (fencing, self-locking doors, sensors, security cameras, safety personnel, etc.).
- Researchers should be involved in the planning and design process of a greenhouse facility, since they have the most profound knowledge of the biological aspects of the work to be performed within that facility.
- The site of the facility should be chosen carefully, ideally in an environment that provides the lowest chance of survival and spread of escaped organisms.
- The most suitable greenhouse design offers good security, is long-lasting, easy to clean, withstands repeated disinfection and minimizes hiding places for pests and other organism

Physical containment in the greenhouse

Physical containment is achieved through facility design and equipment. Choices in the type of glazing, sealing, screening, air flow system, and other features all affect the degree to which a greenhouse is capable of isolating transgenic plants, plant parts, and associated organisms from the surrounding environment. These systems are also effective in keeping unwanted pests out of the greenhouse.

Table 3: Physical containment systems recommended for green house facility

System	Recommended specification
Glazing	The type of glazing most commonly used consists of single panes of tempered glass installed by lapping each pane over the one below. The care taken in installing and maintaining the glazing determines its overall effectiveness
Caulking and sealing	Adequate level of containment may require addition of caulk. Caulking materials are commonly used to seal glass panes, sills, and small openings in and around greenhouse structures. Caulking and sealing restricts the passage of insects and assists with temperature control within the greenhouse.
Screening	When properly sized, installed, and maintained, screen can keep pests and pollinators out of a greenhouse or, conversely, keep experimental organisms in. The integrity of a screening system is determined by several factors including the nature of the material, the size and morphology of the insects being excluded, the hole shape and size, and the air pressure applied on either side of the screen. The maximum hole size generally capable of restricting certain insect species is shown in Table 4.
Negative air pressure	Containment of airborne pollen, spores, and insects is a significant challenge. One strategy to help achieve it is to create negative air pressure within a facility. Negative pressure exists when the amount of air exiting a space exceeds the air intake. Negative pressure bench-top chambers can increase containment of pathogens and insects within greenhouses, screenhouses, and laboratories. A chambered wood and clear plastic box fitted with a blower and filtration system can produce negative pressure on a small scale and at a relatively low cost (Fig. 1).
Cages	Insect cages, when properly used, can increase the containment level of a particular experiment as long as the factors listed above pertaining to screen characteristics and sizing is respected. Fig. 2 is an example of a type of cage available from biological and greenhouse supply companies.

Table 4: Mesh sizes for insect containment

Insect type (adult)	Screen hole size		
	Mesh	Microns	Centimeters
Leaf miners	40	640	0.0625
Leaf white flies	52	460	0.045
Melon aphids	78	340	0.0325
Flower thrip	132	190	0.01875

Source: Adapted from Traynor *et al*., 2001

Fig 1. Negative pressure bench-top
Traynor et al., 2001

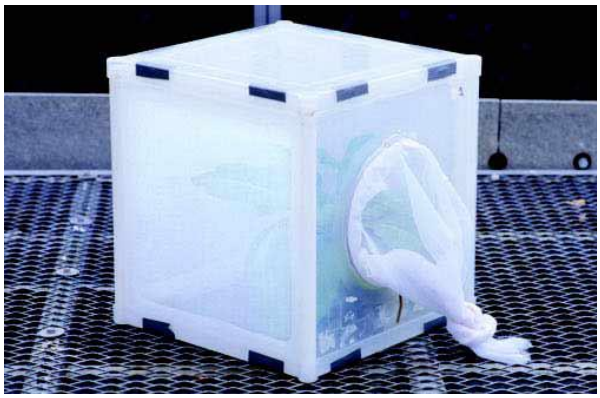

Fig 2. Bugdorm™ insect cage

3.2. Containment of Genetically Modified Micro-organism (GMM)

Specific requirements exist for the risk assessment and containment measures when work with GM M is performed. For the scope of this Guide, micro-organisms shall be defined as "any microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material, including viruses, viroids, animal and plant cells in culture" (EU, 1998). This definition, therefore, includes bacteria, fungi, protozoans, algae and viruses as well as eukaryotic cell cultures, amongst others.

The general containment strategies and procedures described above also applies to micro-organisms. The characteristics of each GMM operation should be evaluated and result in a risk classification, which then dictates the containment measures required to ensure the protection of human health and the environment. In cases of uncertainty regarding the risk classification of a GMM operation higher containment measures, corresponding to a higher risk classification, should be applied. The ultimate result of such a classification is the assignment of the operation to one of the four risk groups described in Table 5.

Table 5: Risk Levels of Genetically Modified Micro-organism

Sn	Risk group	Description
1	Class 1	Activities of no or negligible risk, that is to say activities for which level 1 containment is appropriate to protect human health as well as the environment.
2	Class 2	Activities of low risk, that is to say activities for which level 2 containment is appropriate to protect human health as well as the environment.
3	Class 3	Activities of moderate risk, that is to say activities for which level 3 containment is appropriate to protect human health as well as the environment.
4	Class 4	Activities of high risk, that is to say activities for which level 4

		containment is appropriate to protect human health as well as the environment.

Source: FAO/KAKOLI

The assessment should also take into account the disposal of waste and effluents, and establish adequate safety measures to control these emissions. The containment levels and physical containment measures (often referred to as biosafety levels), which are appropriate for and correspond to each of the four risk classes described above, are described in detail in Annex 4. In addition to the physical containment measures, principles of good laboratory practice should be put in place and followed by all staff involved with the operation. Guidance for such principles is provided in Annex 1.

Furthermore, considerations concerning the characteristics of the likely receiving environment in case of an accident, the scale of the operation and employment of non-standard operations or equipment may alter the risk class of the operation and similarly affect the containment measures that need to be in place to control that risk level.

3.3. Containment of genetically modified animals

As for GM micro-organisms and GM plants, GM animals require specific considerations regarding the risk assessment and the appropriate containment measures. For the scope of this document, animals shall be defined as all motile, heterotrophic organisms, including vertebrates, invertebrates (e.g. insects) and other multicellular organisms. The first activity the responsible competent authority should perform in the case of GM animals is to check whether the experimenter, institution or organization has the approval of the local animal ethics/welfare committee for dealing with the animal species and the attempted trait modification. If this approval is not granted, the research should be kept in abeyance.

To date, genetic modification of animals has a much lower importance than genetic modification of plants, especially in the field of agriculture: so far, no GM animal with a proposed use in agriculture has been granted approval for market release and commercialization.

The steps towards successful GM animal containment are the same as those outlined above in sections 3.1 and 3.2 on GM plants and GM micro-organisms, respectively. First, a risk assessment is performed to evaluate the potential hazards, both to human health and the environment, of the planned GM animal operation Subsequently, the GM animal operation is classified into one of four risk classes (biosafety levels), each of which requires a specific set of containment measures to minimize the risk of adverse effects on human health and the environment.

Special attention should be paid to the following points:

- potential disturbing effects of GM animals on ecosystems, especially if the GM animal has selective advantages over naturally-occurring relatives;
- invasiveness of non-indigenous GM species that occupy the niche or prey upon indigenous species;

- altered consummation behaviour of GM animals with effects on plant/animal life in the ecosystem;
- expression of biologically active compounds with possible implications for interacting species or human health (biopharming).

The detailed containment measures for the four GM animal biosafety levels are listed in Annex 5.

3.4. Biological containment

Biological containment strategies are highly useful for complementing physical containment measures and thus ensuring effective containment of GMOs. As pointed earlier, layering of physical and biological containment measures is considered a most efficient means of achieving containment. Biological containment refers to all measures that directly target the organism being worked on with the aim of preventing sexual or vegetative reproduction and reducing its capability of transgene spread and dissemination, instead of simply providing the physical barriers that contain it in a given area. This can include specific agricultural, horticultural or other work techniques as well as genetic manipulation of the organism to alter its dissemination abilities. These techniques are not only important for research under contained conditions, e.g. in laboratories and greenhouses, but also at later stages of GMO development and commercialization, such as confined field trials or even at the market release stage. Some of the common biological containment techniques are listed in Table 6.

Table 6: Examples of common biological containment techniques

Agricultural management strategies	**Genetic modification/ breeding strategies**	**Strategies for microorganism/insects**
reproductive isolation by removal of flowers prior to anthesis (pollen shed)	use male-sterile lines, or sterile triploid lines or interspecific hybrids	avoid creating aerosols when working with micro-organisms;
cover flower or seed heads (bagging) prior to pollen or seed release (Fig 3.1 and 3.2).	introduce the transgene into the chloroplast genome; chloroplasts are usually maternally inherited, i.e. no transgene spread via pollen takes place;	when challenging plants with pathogens: use disabled pathogens, provide isolation distances between infected and healthy plants, and eliminate vectors that could transfer the pathogen;
ensure temporal isolation from sexually compatible relatives, i.e. grow experimental plants in such a way that flowering takes place at different times than that of sexually compatible relatives in the receiving environment.	employ cleistogamy, i.e. flowers that do not open, resulting in self-pollination;	for insects: use flight-impaired, sterile strains, conduct experiments at time of year or location where survival of escaped organisms is impossible, or choose organisms that have an obligatory relation with the test plant and no other species in the receiving environment
stop experiments and destroy plant material prior to flowering.		

if seeds are produced, stringent measures to collect seed, minimize seed dissemination and prevent seed germination in the receiving environment should be in place.		

Source: Developed from Kakoli/FAO

Fig 3.1 and 3.2: Bagging flowers for biological containment

3.5. Good Laboratory practice (GLP)

Good laboratory practice is a set of standards to describe how research studies should be planned, performed, recorded, archived and reported. Effective containment and many testing procedures are based on sound laboratory management practices. Many guidance documents refer to these practices in general terms as good laboratory practice (GLP). Some countries issue their own versions of the GLP principles based on the OECD Principles of GLP, incorporated as part of national legislations.

The GLP principles describe a set of guidelines for the following: test facility organization and personnel, quality assurance programmes, facilities, apparatus, material and reagents, test systems, test and reference items, Standard Operating Procedures (SOPs), performance of the study, reporting of study results, and storage and retention of records and materials. Please refer to Annex 1 for a summary of GLPs.

4. Design containment facilities

4.1. Laboratory

In designing a laboratory special attention should be paid to conditions that are known to pose safety problems such as unauthorized entry, overcrowding of equipment and experimental material, infestation with rodents and arthropods, formation of aerosols, and outside environmental contamination. Therefore the design features that require special attention are:

- Laboratory location and access
- Surface finishes and casework
- Ventilation and air conditioning
- Containment perimeter
- Laboratory services (i.e., water, drains, gas, electricity, and safety equipment)

The general requirements for the design features are given in Appendix 2.

4.2. Biological safety cabinet

Biological safety cabinets (BSCs) are designed to protect the operator, the laboratory environment and work materials from exposure to infectious aerosols and splashes that may be generated when manipulating materials containing infectious agents, such as primary cultures, stocks and diagnostic specimens. Aerosol particles are created by any activity that imparts energy into a liquid or semi liquid material, such as shaking, pouring, stirring or dropping liquid on to a surface or into another liquid.

Laboratory activities, such as streaking agar plates, inoculating cell culture flasks with a pipette, using a multichannel pipette to dispense liquid suspensions of infectious agents into microculture plates, homogenizing and vortexing infectious materials, and centrifugation of infectious liquids, or working with animals, can generate infectious aerosols. Aerosol particles of less than 5 μm in diameter and small droplets of 5–100 μm in diameter are not visible to the naked eye. The laboratory worker is generally not aware that such particles are being generated and may be inhaled or may cross-contaminate work surface materials. BSCs, when properly used, have been shown to be highly effective in reducing laboratory-acquired infections and cross-contaminations of cultures due to aerosol exposures. Each BSC also protects the environment.

Over the years the basic design of BSCs has undergone several design modifications. A major change was the addition of a high-efficiency particulate air (HEPA) filter to the exhaust system. The HEPA filter traps 99.97% of particles of 0.3 μm in diameter and 99.99% of particles of greater or smaller size. This enables the HEPA filter to effectively trap all known infectious agents and ensure that only microbe-free exhaust air is discharged from the cabinet. A second design modification was to direct HEPA-filtered air over the work surface, providing protection of work surface materials from contamination. This feature is often referred to as product protection. These basic design concepts have led to the evolution of three classes of BSCs. The type of protection provided by each is set out in Table 5.

Class I Cabinets

The Class I BSC was the first recognized BSC and, because of its simple design, it is still in wide use throughout the world. It has the advantage of providing personnel and environmental protection and can also be used for work with radionuclides and volatile toxic chemicals. Because unsterilized room air is drawn over the work surface through the front opening, it is not considered to provide consistently reliable product protection. Fig. 4 provides a schematic diagram of a Class I BSC. The air from the cabinet is exhausted through a HEPA filter

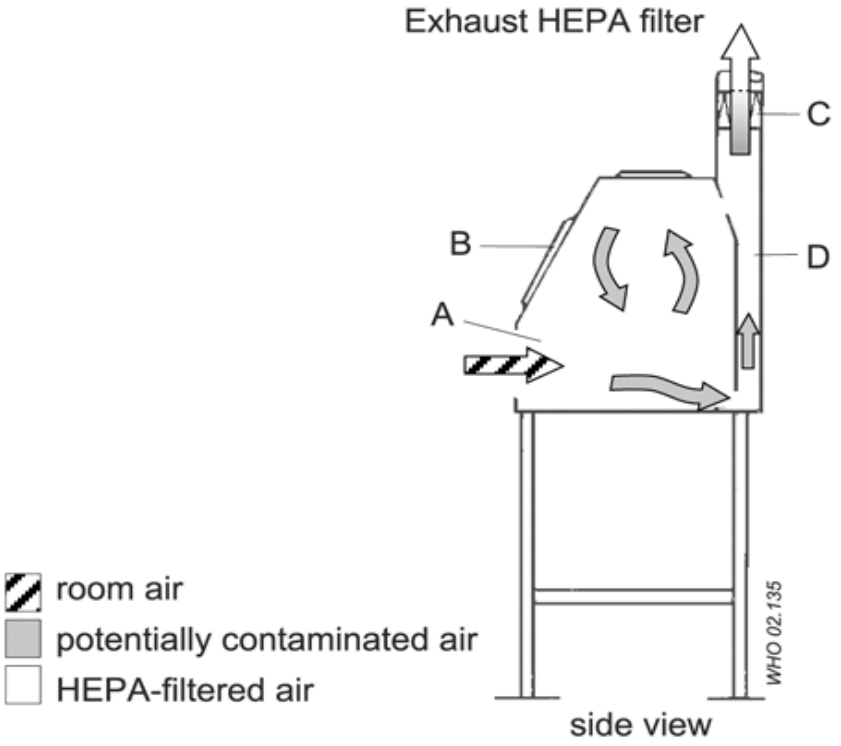

Fig. 4. Schematic diagram of a Class I biological safety cabinet. A: front opening, B: sash, C: exhaust HEPA filter, D: exhaust plenum.

Class II biological safety cabinets

As the use of cell and tissue cultures for the propagation of viruses and other purposes grew, it was no longer considered satisfactory for unsterilized room air to pass over the work surface. The Class II BSC was designed not only to provide personnel protection but also to protect work surface materials from contaminated room air. Class II BSCs, of which there are four types (A1, A2, B1 and B2), differ from Class I BSCs by allowing only HEPA-filtered (sterile) supply air to flow over the work surface. The Class II BSC can be used for working with infectious agents in Risk Groups 2 and 3. Class II BSCs can be used for working with infectious agents in Risk Group 4 when positive pressure suits are used.

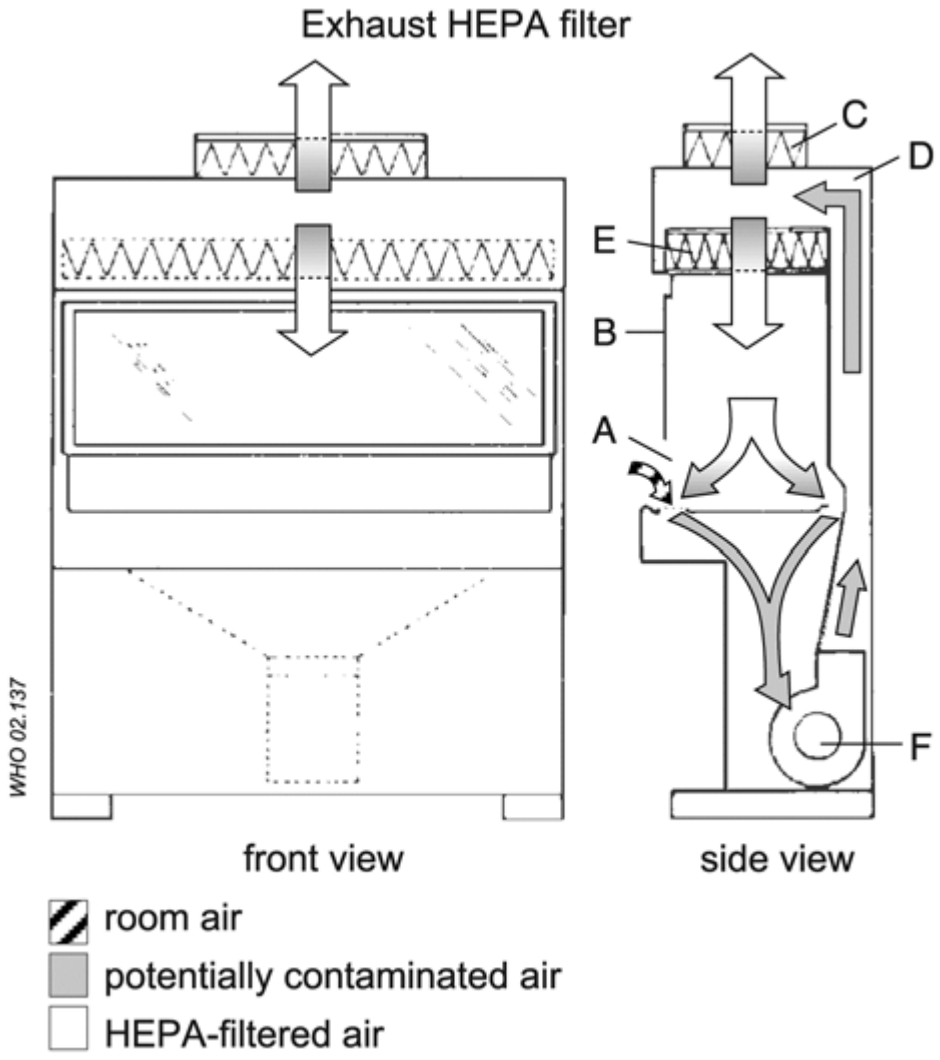

Fig. 5. Schematic representation of a Class IIA1 biological safety cabinet. A: front opening, B: sash, C: exhaust HEPA filter, D: rear plenum, E: supply HEPA filter, F: blower.

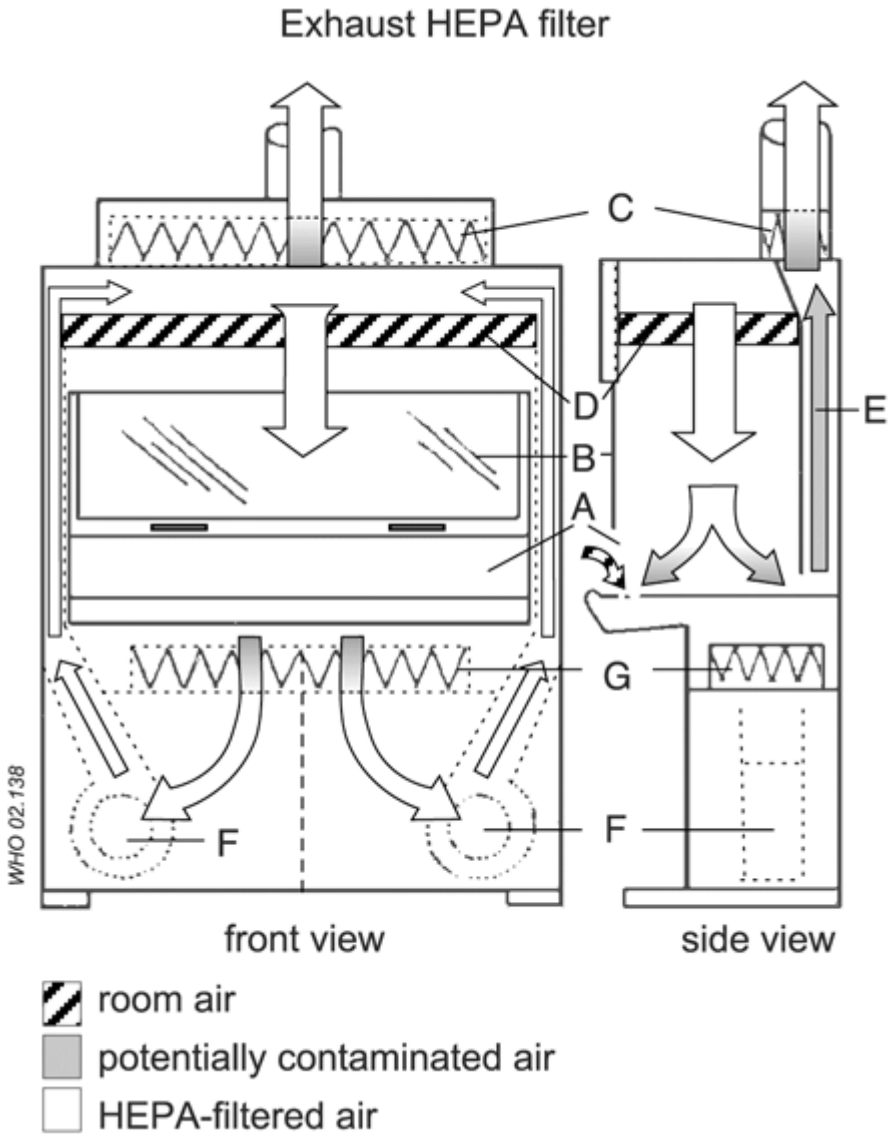

Fig. 5. Schematic diagram of a Class IIB1 biological safety cabinet. A: front opening, B: sash, C: exhaust HEPA filter, D: supply HEPA filter, E: negative pressure exhaust plenum, F: blower, G: HEPA filter for supply air. Connection of cabinet exhaust to building exhaust air system is required.

Class III biological safety cabinet

This BSC type (Fig. 6) provides the highest level of personnel protection and is used for Risk Group 4 agents. All penetrations are sealed "gas tight". Supply air is HEPA-filtered and exhaust air passes through two HEPA filters. Air flow is maintained by a dedicated exhaust system exterior to the cabinet, which keeps the cabinet interior under negative pressure (about 124.5 Pa). Access to the work surface is by means of heavy duty rubber gloves, which are attached to ports in the cabinet. The Class III BSC should have an attached pass-through box that is sterilizable and is equipped with HEPA-filtered exhaust. The Class III cabinet may be connected to a double-door autoclave used to decontaminate all materials entering or exiting the cabinet. Several glove

boxes can be joined together to extend the work surface. Class III BSCs are suitable for work in Biosafety Level 3 and 4 laboratories.

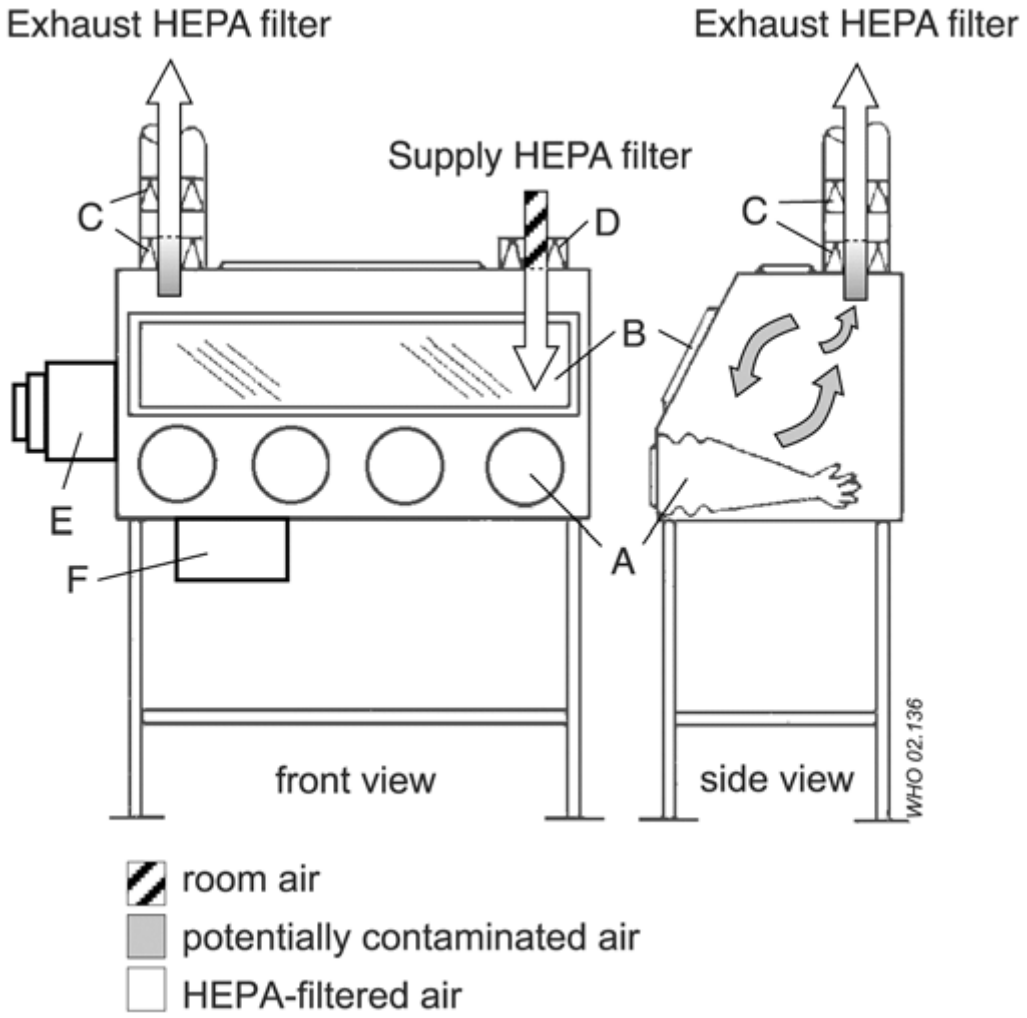

Fig. 6. Schematic representation of a Class III biological safety cabinet (glove box). A: glove ports for arm-length gloves, B: sash, C: double exhaust HEPA filters, D: supply HEPA filter, E: double-ended autoclave or pass-through box, F: chemical dunk tank. Connection of cabinet exhaust to an independent building exhaust air system is required.

Horizontal Laminar Flow "Clean Bench"

Horizontal laminar flow clean air benches are not BSCs. They discharge HEPA filtered air across the work surface and toward the user. These devices only provide product protection not for personnel. These benches should never be used as a substitute for a biological safety cabinet in research, biomedical or veterinary laboratories and/or applications.

Vertical Laminar Flow "Clean Bench"
Vertical laminar flow clean benches also are not BSCs. While these units generally have a sash, the air is usually discharged into the room under the sash, resulting in the same potential problems as the horizontal laminar flow clean benches.

4.3. Greenhouse/ Screen house

Designing new facility *level 3*
A new greenhouse intended for research with GMOs should be designed and built to maintain containment for the life of the facility. A greenhouse built to BL1-P or BL2-P containment standards costs little more than a standard research greenhouse. Because of more stringent design requirements, greenhouses built to BL3-P or BL4-P specifications will cost significantly more than conventional facilities. For the same reason, a qualified and experienced team of designers must render the detailed plans for such facilities.

Framing materials *level 4*
Typical construction styles for contained research include even-span with a standard peak, Venlo, and ridge and furrow with gutter connects. Roof styles include the standard peaked, as well as arched, mansard, and Quonset-style. Modern greenhouse structures are framed with aluminum or galvanized steel. Wood and pipe framing are still being used in new construction of some plastic film greenhouses. A reinforced, rigid frame is preferred for BL3-P and required for BL4-P. The latter requires additional strength and rigidity to accommodate the weight of double-paned, break-resistant, sealed glass.

Entry door and locks *level 4*
The choice of greenhouse doors should receive careful consideration since containment and security breaches occur most often at points of entry. Specifications for BL3-P and BL4-P facilities stipulate a double set of self-closing and locking doors. High containment facilities also require one-way emergency exit doors for personnel safety. Traditional cylinder locks offer good security. A double-door entry system, with a dark vestibule sandwiched between the doors, aids in effective insect containment.

Benching *level 4*
Many different types of benching can be found in research facilities, but when building a new high level containment greenhouse, the design and materials should be chosen so as to comply with appropriate biosafety level requirements. Those made of aluminum or galvanized steel provide the longest wear, are easiest to clean, and amenable to installing systems for runoff water collection and treatment

Ventilation, heating and cooling *level 4*
Air conditioning is not strickly mandatory to higher level containment greenhouses; however the loss of cooling efficiency due to required air-handling measures makes it a necessity in most climates.

The exhaust air produced from negative pressure systems must be filtered to prevent contained organisms from exiting. Intake air is also filtered to prevent introduction of organisms from the

environment into the enclosed space. Filter systems can be designed to trap pollen, spores, and other very small particles. High efficiency particulate air (HEPA) filters can remove 0.3 micron and larger particles but still allow gases to transfer across the filter media.

The engineering specifications required for air balancing, ventilating, and cooling BL3-P and BL4-P greenhouses are beyond the scope of this Guide

4.4. Retrofitting conventional greenhouses level 3

Retrofitting a conventional greenhouse to meet BL1-P and BL2-P containment standards is far cheaper than building a new facility. Requirements for meeting BL3-P standards are more extensive and may involve basic structural changes; therefore, retrofitting may not be feasible or cost-effective. Similarly, if a greenhouse is structurally unsound or suspect, rebuilding may be the best option. BL4-P standards require a dedicated, highly engineered, and isolated facility, which excludes the possibility of retrofitting existing greenhouses. Accordingly, this section primarily concerns modifications that would bring a conventional greenhouse up to the containment standards appropriate for the lower biosafety levels.

Existing greenhouse facilities should be carefully inspected to determine if they are suitable for retrofitting. Structurally sound buildings in good condition are often adequate, or nearly so, in terms of containment. Necessary modifications, if any, are usually simple, straightforward, and involve readily available materials. Before deciding to retrofit an existing greenhouse, the cost should first be compared to that of building a new structure. If retrofitting costs fall within 20% of the price of new construction, renovation generally is not recommended. It is advisable to contact a greenhouse builder, engineer, architect, or experienced consultant before proceeding with any major renovation. Upgrades needed to meet specified containment standards are shown in Table 8.

Table 8: Enhanced features for containment green houses and screen houses

Feature	Conventional	BL1	BL2	BL3	BL4
Structure	Framing may be aluminum, steel, wood, or pipe			Rigid, wind resistant frame preferred; internal walls, ceilings, and floors resistant to liquids and chemicals	Reinforced, rigid frame required; walls, floors, and ceilings form sealed internal shell, resistant to liquids and chemicals; see Appendix P for others
Entry	Hinged or sliding entry doors		Locks on entry doors	Double set of self-closing, locking doors	Double set of self-closing, locking doors with air-lock; shower and changing rooms
Glazing	Standard greenhouse glass or plastic material			Laminated, strengthened, sealed	Double-paned, laminated, strengthened, sealed
Screening	If used, standard 30 mesh fly screen	Recommended	30-mesh or higher required	Not permitted	Not permitted
Ventilation	Roof or side vents, fans, cooling pads,			Separate negative pressure system; air	Air-conditioned and HEPA filtered, closely

	fog system, or a combination of these			supply fans with back- flow damper; exhaust air HEPA filtered	monitored negative pressure, no roof or side vent allowed
Benching	Any material; solid or porous bottoms			Seamless water and chemical resistant bench tops	Seamless water and chemical resistant bench tops
Floors	Gravel (most common), soil, or concrete throughout	Impervious walkways recommended	Impervious material; collection of runoff water may be required	Impervious material; for microbes, runoff water collection and decontamination	Sealed floors as part of internal shell; runoff collection and decontamination
Drains	Discharge into groundwater or sanitary/storm sewer			Provision for collection and decontamination of runoff	Runoff collection required, sewer vents filtered
Other	Automatic control and utility systems meet basic operating requirements		Autoclave available	Autoclave within facility; hand washing with hands free on/off; filtered vacuum lines; disinfectant traps for liquid lines	Double-door autoclave; self-contained vacuum system; in-line filters and back-flow protection for all liquid/gas services

Source: Traynor et al., 2001

5. Management/Operational procedures

Containment strategies are effective only when personnel managing the facilities understand and adhere to established procedures for handling genetically modified material. Before entering the premises, all staff working around transgenic organisms should be fully informed about the containment measures applicable to a given research project. Prescribed procedures and practices should be appropriate for the assigned biosafety level; those that appear excessive for the needed level of containment may discourage compliance.

Access

Access to laboratories and facilities handling GMOs or infectious agents is restricted, regardless of the biosafety level. At BL1 and BL2, access is restricted at the discretion of the facility manager or PI when experiments are in progress. At BL3 and BL4, the manager, in consultation with the PI should determine access authorization on an individual basis. At BL3 and BL4, a facility staff should accompany maintenance personnel and visitors. An entry and exit logbook is required at BL4 to provide details of names, dates and times of entry and exit by each individual.

Signs

No special signs are required for BL1 containment facilities. Entryways into BL2 and higher facilities should be posted with signs indicating that access is limited to authorized personnel only. The internationally recognized biohazard warning symbol and sign (Figure 2) must be displayed on doors of rooms handling organisms classified as BL2 and above. If the experiment uses organisms that pose a risk to the local ecosystem or agriculture, a sign stating so must be placed on the access doors to the laboratory and research facilities. A description of the potential

risk may be posted on the restricted access sign as long as this is not confidential information. Experimental materials and chemicals within the facility must also be marked with appropriate signs.

Disinfection, Sterilization and Hygiene

A basic knowledge of disinfection, sterilization and hygiene is crucial for biosafety in the laboratory and research facilities. The specific requirements for decontamination for biosafety will depend on the type of experimental work and the nature of the infectious agent(s) being handled. Procedure of application of disinfectants varies with material and manufacturer. Therefore, usage should follow manufacturers' specifications. Pre-cleaning, to remove dirt, organic matter and stains, is essential to achieve proper disinfection or sterilization. Disinfection can be done using chemical germicides, such as chlorine, formaldehyde, phenolics, ethanol/ alcohol and hydrogen peroxide. Heat is the most commonly used method of sterilization. "Moist" heat is most effective when used in the form of autoclaving. Dry heat can also be used for sterilization.

Handling and Transfer of Materials

Handling and transfer of GM materials or infectious agents for levels BL2-4 should be done in non-breakable containers. For BL3 and BL4 containment, additional sealed secondary container for movement of experimental materials is required. To avoid accidental leakage or spillage, secondary containers, such as boxes, should be used, fitted with racks so that the specimen containers remain upright. The secondary containers may be of metal or plastic, should be autoclavable or resistant to the action of chemical disinfectants, and the seal should preferably have a gasket. They should be regularly decontaminated. The exterior surface of the secondary chamber should be decontaminated either chemically or in a fumigation chamber if the same plant, animal, host, or vector is present within the effective dissemination distance of the propagules/ progenies of the experimental organism.

Storage

GMOs or infectious materials should be stored in a lockable facility so as to minimize handling in unconfined spaces. The GMO should be clearly identified and labeled to distinguish it from other stored materials, and preferably stored separately from related species to avoid contamination. Access to the storage facility should be limited to authorized individuals.

Personnel Safety

Personnel working in the containment facilities should adhere to good laboratory practices (GLPs) and avoid misuse of equipment that causes the majority of laboratory accidents, injuries and work-related hazards. Thus, a safety-conscious staff well informed about the recognition and control of laboratory hazards, personal protective equipment and clothing, is key to the prevention of laboratory-acquired infections, incidents and accidents. For this reason, continuous in-service training in safety measures is essential.

Records

The extent of record keeping required for research using GMO is commensurate with the level of biosafety. Records of experiments in progress must be kept for all biosafety levels. At BL2 and higher, additional records must be kept of all plants, animals and their associated organisms

entering or leaving the containment facility. A record of the dates and times of personnel visits must be kept for BL4 facilities.

Termination and Disposal
To prevent the unintended survival of GMOs or infectious agents outside the contained environment, all experimental materials must be rendered biologically inactive (devitalized) before disposal. Devitalization can be achieved by burning, incineration, autoclaving or chemical treatment. Termination procedures for the safe disposal of soil, plant and animal material should be part of the experimental plan for a research project.

Training
Continuous in-service training in safety measures is essential for effective containment. An effective safety programme begins with the laboratory managers, who should ensure that safe laboratory practices and procedures are integrated into the basic training of employees. Training in safety measures should be an integral part of new employees' introduction to the laboratory.

Health and Medical Surveillance
The employing authority is responsible for ensuring that there is adequate surveillance of the health of laboratory personnel. The objective of such surveillance is to monitor for occupationally acquired diseases. Appropriate activities to achieve these objectives are to:

- Provide active or passive immunization where indicated
- Facilitate the early detection of laboratory-acquired infections
- Exclude highly susceptible individuals (such as pregnant women) from highly hazardous laboratory work
- Provide effective personal protective equipment and procedures.

Inspection
Inspections should be conducted on proposed new facilities, and regularly and also whenever new types of experimental materials are brought into an established facility. Biosafety Inspectors from the Ministry responsible for Agriculture as well as any other agents authorized by Minister responsible for biosafety should conduct this inspection for adequacy and compliance with the Terms and Conditions of authorization. A facility inspection checklist for containment of GMOs has been provided as Annex 6.

Contingency plans and emergency procedures
The Principal Investigator (PI) will establish a contingency plan for actions to be taken in case of emergency, or of unauthorized or accidental release of GM material or infectious agents (Appendix 4). A written contingency plan for dealing with laboratory and animal facility accidents is a necessity in any facility that works with or stores Risk Group 3 or 4 organisms.

Annexes

Annex 1: Good Laboratory Practice

The following points should be considered for every operation with GMOs and within containment facilities (EU, 1998; see also WHO, 2004):

- keep workplace and environmental exposure to any GMM to the lowest practicable level;
- exercise engineering control measures at source and to supplement these with appropriate personal protective clothing and equipment when necessary;
- test adequately and maintain control measures and equipment;
- test, when necessary, for the presence of viable process organisms outside the primary physical containment;
- provide appropriate training of personnel;
- establish biological safety committees or subcommittees, if required;
- formulate and implement local codes of practice for the safety of personnel, as required;
- where appropriate to display biohazard signs;
- provide washing and decontamination facilities for personnel;
- keep adequate records;
- prohibit eating, drinking, smoking, applying cosmetics or the storing of food for human consumption in the work area;
- prohibit mouth pipetting;
- provide written standard operating procedures, where appropriate, to ensure safety;
- have effective disinfectants and specified disinfection procedures available in case of spillage of GMMs;
- provide safe storage for contaminated laboratory equipment and materials, when appropriate.

In addition to these principles, the appropriate containment measures for the risk class of the operation should be in place in order to asssure protection of human health and the environment.

The containment measures applied shall be periodically reviewed by the user to take into account new scientific or technical knowledge relative to risk management and treatment and disposal of wastes.

Annex 2: General requirements for laboratory design and physical requirements (adopted from HC, 2004,……..)

Laboratory Location and Access

Containment Level				**Laboratory Location and Access**
1	**2**	**3**	**4**	
●	●	●	●	Separated from public areas by door.
●	●	●	●	Access limited to authorized personnel.
●	●	●	●	Laboratory room doors to have appropriate signage (e.g., biohazard sign, containment level, contact information, entry requirements).
●	●	●	●	Size of door openings to allow passage of all anticipated equipment.
●	●	●	●	Doors to the containment laboratory lockable (this does not apply to areas within the containment laboratory).
		●	●	Doors to provide restricted access by installation of a controlled access system (e.g., card key) or equivalent.
		○	●	Electronic locking systems to be backed up with a physical key-lock system.
	●	●	●	Office areas to be located outside of containment laboratory. Paperwork stations for data collection can be within containment laboratory provided they are located away from laboratory work areas.
	●	●	●	Entry to laboratory to be provided via a waiting room.
		●	●	Waiting room door(s) located between the clean and dirty change rooms not to be opened simultaneously with either the containment laboratory door or the clean change entry door. (Interlock, visual or audible alarms, or protocols are all acceptable means.)
		●	●	Waiting room door(s) located between the clean and dirty change rooms not to be opened simultaneously with either the containment laboratory door or the clean change entry door (interlock only).
		●	●	Interlocked doors, if present, to have manual overrides for emergency exit.
		●	●	Entry to laboratory zone to be provided with clothing change areas separating personal and laboratory clothing dedicated to that zone (i.e., "clean" change area separated from "dirty" change area).

		●	●	Exit from laboratory to be provided with a walk-through shower on the containment barrier (i.e., between “dirty” and "clean" change waiting rooms). (CL3 laboratories manipulating organisms, such as HIV, that are not infectious via inhalation, are not required to fulfill this criterion.)
			●	Entry to laboratory to be provided via waiting room with airtight doors (e.g., inflatable or compression seal); for laboratories using only a Class III BSC biological safety cabinet line, airtight doors are not required.
			●	Entry to laboratory zone to be provided with a suit change area, a chemical shower on the containment barrier (i.e., between the laboratory and suit change area) and water shower on exit from the zone (i.e., between "dirty" and "clean" change areas); for laboratories using only a Class III biological safety cabinet line, suit change area and chemical shower are not required.
		●	●	Containment laboratories to be located in close proximity to supporting mechanical services to limit the amount of potentially contaminated services.
		○	●	Containment laboratories to be located away from external building envelope walls.
		○	○	A laboratory support area to be provided adjacent to the containment facility for all supporting laboratory manipulations.

Surface (i.e., floors, walls, ceilings, sealants) Finishes and Casework

Containment Level				**Surface (i.e., floors, walls, ceilings, sealants) Finishes and Casework**
1	**2**	**3**	**4**	
●	●	●	●	Doors, frames, casework and bench tops to be nonabsorptive (i.e., the use of organic materials should be avoided).
●	●	●	●	Working surfaces of bench tops to be non-absorptive.
●	●	●	●	Surfaces to be scratch, stain, moisture, chemical and heat resistant in accordance with laboratory function.
●	●	●	●	Surfaces to provide impact resistance in accordance with laboratory function.
	○	●	●	Surfaces to be continuous and compatible with adjacent and overlapping materials (i.e., to maintain adhesion and a continuous perimeter); wall and floor welded seams are acceptable in level 3 laboratories.
		●	●	Continuity of seal to be maintained between the floor and wall (a continuous cove floor finish up the wall is recommended).
		●	●	Interior surfaces to minimize movement of gases and liquid through perimeter membrane.
○	●	●	●	Interior coatings to be gas and chemical resistant in accordance with laboratory function.
●	●	●	●	Interior coatings to be cleanable.
			●	Structural stability to withstand 1.25 times maximum design pressure under supply and exhaust fan failure conditions (i.e., no wall distortion or damage).
●	●	●	●	Bench tops to have no open seams.
○	○	○	●	Benches, doors, drawers, door handles, etc. to have rounded rims and corners.
●	●	●	●	Reagent shelving to be equipped with lip edges.

Heating, Ventilation and Air Conditioning (HVAC)

Containment Level				**HVAC**
1	**2**	**3**	**4**	
	○	●	●	100% outside air to be supplied.
		●	●	Directional inward airflow provided such that air will always flow towards areas of higher containment.
		●	●	Visual pressure differential monitoring devices to be provided at entry to containment laboratory.
			●	Room pressure differential monitoring lines penetrating the containment barrier to be provided with filters of efficiency filtration.
		●	●	Alarm (visual or audible) to be provided in the laboratory and outside laboratory area (i.e., to warn others and maintenance personnel) to signal air handling systems failure.
		●	●	Where determined necessary by a local risk assessment, supply air duct to be provided with backdraft protection
			●	Supply air to be filtered.
		●	●	Supply air system to be independent of other laboratory areas. (For laboratories manipulating organisms, such as HIV, that are not infectious via inhalation this criterion is only recommended.)
		●	●	Supply air system to be interlocked (i.e., fans, dampers, electrical) with exhaust air system, to prevent sustained laboratory positive pressurization.
		●	●	Exhaust air to be filtered. (laboratories manipulating organisms, such as HIV, that are not infectious via inhalation are not required to fulfil this criterion.)
			●	Exhaust air to be passed through two stages of filtration.
		●	●	Filters installed into the supply and exhaust system to conform to the standard requirements.

Containment Perimeter

Containment Level				**Containment Perimeter**
1	**2**	**3**	**4**	
●	●			Autoclave or other acceptable means of waste treatment/disposal to be provided.
		●	●	Double-door barrier autoclave with bioseal to be located on containment barrier; body of autoclave to be preferably located outside of containment for ease of maintenance.
		●		Barrier autoclave to be equipped with interlocking doors, or visual or audible alarms to prevent both doors from opening at the same time.
			●	Barrier autoclave to be equipped with interlocking doors, and visual or audible alarms to prevent both doors from opening at the same time.
●	●	●	●	For materials that cannot be autoclaved (e.g., heat sensitive equipment, samples, film) other proven technologies for waste treatment (e.g., incineration, chemical, or gas) to be provided at containment barrier.
		●	●	All penetrations to be sealed with nonshrinking sealant at containment barrier.
		●	●	All conduit and wiring to be sealed with nonshrinking sealant at the containment barrier.
●	●			Windows, if they can be opened, to be protected by fly and pollen screens.
		●	●	Windows positioned on containment barrier to be sealed in place; window glazing material to provide required level of security.
		○	○	Observation windows to be installed on containment barrier.

Laboratory Services (i.e., water, drains, gas, electricity, and safety equipment)

Containment Level				**Laboratory Services (i.e., water, drains, gas, electricity, and safety equipment)**
1	**2**	**3**	**4**	
●	●			Hooks to be provided for laboratory coats at laboratory exit; street and laboratory clothing areas to be separated.
●	●	●	●	Handwashing sinks to be located near the point of exit from the laboratory or in waiting room.
	○	●	●	Handwashing sinks to be provided with "hands-free" capability.
		●	●	Other primary containment devices to be provided.
●	●	●	●	Emergency eyewash facilities to be provided in accordance with applicable regulations
●	●	●	●	Emergency shower equipment to be provided in accordance with applicable regulations
		●	●	Domestic water branch piping serving laboratory area(s) to be provided with backflow prevention, and isolation valve, to be located in close proximity to the containment barrier.
		●		Drain lines and associated piping (including autoclave condensate) to be separated from lower containment laboratory areas and to go directly to main building sanitary sewer at point of exit from building (downstream of all other connections).
			●	Drain lines and associated piping (including autoclave condensate) to be separated from areas of lower containment and to be connected to an effluent sterilization system.
			●	Drains connected to effluent sterilization to be sloped towards sterilization system to ensure gravity flow; consideration should be given to the installation of valves to isolate sections of piping for *in situ* decontamination; the effluent sterilization system (e.g., piping, valves, tank) to be heat and chemical resistant consistent with application.
		●	●	Autoclave condensate drains to have a closed connection.
		●	●	Drainage traps to be provided to required deep seal depth in consideration of air pressure differentials.
		○	●	Floor drains not to be provided, except when essential (e.g., body shower and animal rooms).
			●	Plumbing vent lines (including effluent sterilization system) to be provided with filter of efficiency and provided with a means of

				isolation and decontamination.
		●		Plumbing vent lines to be independent of lower containment plumbing vent lines, or combined with lines from lower containment when provided with a filter of efficiency.
		○	●	Compressed gas cylinder(s) to be located outside the laboratory.
			●	Laboratory supply gas piping (e.g., carbon dioxide, compressed air) to be provided with backflow prevention.
		●	●	Portable vacuum pump to be provided in the laboratory. Internal contamination of vacuum pump to be minimized
			●	Compressed breathing air to be provided to positive-pressure personal protective equipment (i.e., for connection to the air hose of suits), equipped with breathing air compressors and back-up cylinders (sufficient for 30 minutes per person); air hose connections to be provided in all areas where suits are worn, including chemical shower and suit change room.
		●	●	Emergency lighting to be provided.
		●	●	Life safety systems, lighting, security systems and other essential equipment to be supported with emergency back-up power.
		●	●	Circuit breakers to be located outside biocontainment area.
○	○	●	●	Laboratory to be equipped with a communication system between containment area and outside support area.
		●	●	System (e.g., fax, computer) to be provided for electronic transfer of information and data from laboratory area to outside laboratory perimeter. (Note: paperwork from the containment laboratory may be removed after appropriate decontamination, i.e., autoclaving, irradiation, microwaving; such practices are generally not recommended for use on a routine basis).
			●	Work area to be monitored (e.g., closed circuit TV) from outside laboratory perimeter (e.g., security/biosafety office).

Storage Facility

Containment level				Storage facility
1	2	3	4	
●	●	●	●	The store is clean, sanitized, intact and labeled
●	●	●	●	Containers are sealed and labeled
○	●	●	●	Multiple samples of one transgenic materials be stored separately in sealed containers
○	●	●	●	Multiple samples of different transgenic materials stored separately in sealed containers
●	●	●	●	Forms for storage inspection are available and recorded accordingly
●	●	●	●	Containment of accidentally released transgenic materials is effected
●	●	●	●	Protocols for notification of accidental release of transgenic materials to relevant parties are filled in the record of corrective action
○	●	●	●	Record of storage inspection retained in compliance document binder
●	●	●	●	Doors to the store are lockable
●	●	●	●	Store windows are closed and locked
●	●	●	●	Store be sanitized immediately after the period of storage
●	●	●	●	Disposed transgenic materials destroyed by dry heat/stem heat/crushing/burning/autoclaving/chemical treatment
○	●	●	●	Compliance document binder available to TPRI inspectors upon request

Annex 3: Containment measures for Greenhouse activities with genetically modified plants

Containment measure	Containment level			
	1	2	3	4
Green house access				
Limited or restricted	Yes	Yes	Yes	Yes
Access managed by responsible individual	/	/	/	Yes, access through secure, locked doors
Warning of potential hazards prior to entering	/	/	/	Yes
Entrance only through clothing change and shower room	/	/	/	Yes, shower each time greenhouse is left
Training prior to access	Yes	Yes	Yes	Yes
Records				
Record of current experiments	Yes	Yes	Yes	Yes
Record of all organisms that are brought into or removed from the greenhouse	/	Yes	Yes	Yes, plus all materials
Reporting of any accident involving release of GMO s	/	Yes	Yes	Yes
Record of persons entering/ exiting the greenhouse	/	/	/	Yes
Decontamination and in activation				
GMO s rendered biologically inactive before disposal	Yes	Yes	Yes, autoclaving recommended	Yes, by autoclaving
Decontamination of run-off water	/	Recommended	Yes	Yes
Decontamination of equipment	/	/	Yes	Yes
Control of undesired species				
Program to control undesired species	Yes	Yes	Yes	Yes, chemical control
Anthropods and motile macro-organisms kept in cages; precautions to minimize escape	Yes	Yes	Yes	Yes
Concurrent experiments conducted				
Experiments with a lower biosafety level can be conducted concurrently	Yes	Yes	Yes	Yes
Greenhouse design				
Greenhouse floor	Gravel or other porous material	Impervious material. Gravel under benches and soil beds acceptable.	Impervious material with collection of run-off water	Walls, roof and floor form sealed, resistant internal shell
Windows and wall/roof openings	May be open for ventilation	May be open for ventilation	Closed and sealed	Closed and sealed
Gazing	/	/	Resistant to breakage	Resistant to breakage

Screens	Recommended	Required	/	/
Greenhouse isolation and entry	/	/	Closed, self-contained structure, self-closing Locking doors	Closed, self-contained structure, self-closing Locking doors
Fencing and security	/	/	Yes	Yes
Internal walls, ceilings and floors	/	/	Resistant to penetration	Resistant to penetration
Bench top material	/	/	Impervious, resistant Surfaces	Impervious, resistant Surfaces
Hand washing sink/shower	/	/	Sink, automatically operated	Shower
Changing rooms	/	/	/	Yes, outer and inner and shower
Airlock	/	/	/	Yes, for material passage
Autoclaves				
An autoclave should be Available	/	Yes	Yes	Yes, double door
Air ventilation system				
Minimize entrance of Anthropods	/	Yes	/	/
Individual supply and exhaust systems	/	/	Yes	Yes
Negative pressure	/	/	Yes	Yes
HEPA filtering of exhaust air	/	/	Yes	Yes
HEPA filtering of ventilation Lines	/	/	Yes, on vacuum lines	Yes
Signs				
Signs indicating that a restricted experiment is in progress	/	Yes	Yes	Yes
Signs indicating the presence of organisms with potential for environmental damage	/	Yes, if applicable	Yes, if applicable	Yes, if applicable
Sign indicating risks to human health (biohazard sign)	/	Yes, if applicable	Yes, if applicable	Yes, if applicable
Transfer Materials				
Transfer of viable organisms to/from the facility	/	Transfer in a closed, non-breakable container	Transfer in a sealed Secondary container	Transfer in a sealed Secondary container
Transfer of materials and supplies	/	/	/	Transfer through Autoclave airlock or Fumigation chamber
Protective clothing				
Disposable clothing should be worn in the greenhouse	/	/	Yes, if considered necessary	Yes may be disposable
Exchange of street clothing to complete laboratory clothing	/	/	/	Yes
Protective clothing removed before exiting the greenhouse and decontaminated	/	/	Yes	Yes. by autoclaving
Greenhouse practice manual				
A greenhouse practices manual should be prepared and adopted	/	Yes	Yes	Yes
Other				

Hand wash upon exiting the greenhouse	/	/	Yes	/
Shower upon exit	/	/	/	Yes
Procedures performed to minimize creation of aerosols/splashes	/	/	Yes	Yes

Annex 4: Containment measures for research involving genetically modified microorganism

Containment measure	Containment level			
	1	2	3	4
Laboratory isolation	not required	not required	required	required
Fumigation	not required	not required	required	required
Equipment				
Surface impervious to water and resistance 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 & walls
Entry to laboratory viaairlock	not required	not required	required where and to extent the risk assessment shows it is required	required
Negative pressure relative to the pressure of the immediate surroundings	not required	required where and to extent the risk assessment shows it isrequired	required	required
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
Microbiological safety cabinet/enclosure	not required	required where and to extent the risk assessment shows it is required	required and all procedures with infective materials required to be contained within a cabinet/ enclosure	Class III cabinet Required
Autoclave	Required on site	Required in the building	Required in the laboratory suite	double ended autoclave required in laboratory
System of work				
Access restricted to authorized personnel only	not required	required	required	required via airlock key procedure
Specific measures to control aerosol dissemination	not required	required so as to minimize	required so as to prevent	required so as to prevent
Shower	not required	not required	required where and to extent the risk assessment shows it is required	Required
Protective clothing	Suitable protective Clothing required	Suitable protective Clothing required	Suitable protective Clothing required; Footwear required and to extent the risk assessment shows it is required	Complete change of clothing &footwear required before
Gloves	Not required	required and to extent the risk assessment shows it is required	required	Required

Efficient control of disease vectors (e.g. for rodents and insects) which could disseminate the GMM	required and to extent the risk assessment shows it is required	required	required	required
Specified disinfection procedures in place	required and to extent the risk assessment shows it is required	required	required	required
Waste				
Inactivation of GMMs in effluent from hand washing sinks and showers and similar effluents	Not required	Not required	required and to extent the risk assessment shows it is required	required
Inactivation of GMMs in contaminated material	required by validated means	required by validated means	required by validated means, with waste inactivated in the laboratory suite	required by validated means, with waste inactivated in the laboratory suite
Other measures				
Laboratory to contain its own equipment	Not required	Not required	required so far as is reasonably practicable	required
An observation window or alternative is to be present so that occupants can be seen	required and to extent the risk assessment shows it is required	required and to extent the risk assessment shows it is required	required	required
Safe storage of GMMs	required and to extent the risk assessment shows it is required	required	required	Secure storage required
Written records of staff Training	Not required		required	required

Annex 5: Containment measures for genetically modified animals

Containment measure	Containment level			
	1	2	3	4
Animal facility				
Animals contained in enclosed structure (animal room)	Yes	Yes	Yes	Yes
Interior walls, floors and ceilings impervious and resistant	/	Yes	Yes	Yes
Windows	/	/	/	Yes
Autoclave available	/	/	/	Yes, shower each time greenhouse is left
Self closing doors	/	/	Yes	Yes
Anthropod- proof structure	/	Yes	Yes	Yes
Double barrier between containment area and environment	/	/	Yes	Yes animal area separated from other areas
Necropsy room	/	/	/	Yes
Decontamination of waste and run-off water	/	/	Yes	Yes, by heat or chemical methods
Directional airflow (inwards)	/	/	Yes	Yes
Double HEPA filtering of exhaust air	/	/	Single filter if required	Yes
Exhaust air incinerator	/	/	/	Yes. as alternative to double HEPA filtering
Floor drains with deep traps	/	/	/	Yes
Hand washing sink	/	/	/	Yes, automatically operated
Restraining devices for animals	/	/	/	Yes
Supply water system with backflow preventer	/	/	/	Yes
All utilities, liquid and gas services with backflow preventer	/	/	/	Yes
Ventilation lines with HEPA filters	/	/	/	Yes
Animal facility access				
Individuals under 16 years not permitted	/	/	/	Yes
Containment area locked	Yes	Yes	Yes	Yes
Containment area patrolled or monitored	Yes	Yes	Yes	Yes
Containment building patrolled, with locking access	/	Yes	Yes	Yes
Restricted access, warning of potential hazards	Yes	Yes	Yes	Yes
Entrance/exit through clothing change/shower rooms	/	/	/	Yes
All closures closed when experiment in progress	/	/	Yes	Yes
Decontamination and in activation				
All wastes decontaminated	/	Yes	Yes	Yes
Work surfaces and equipment decontaminated after work	/	/	Yes	Yes
Removal of material	/	/	Special requirement	Only after autoclaving
Chemical disinfectant	/	/	/	Yes, if such suits are

shower for ventilated suits				required
Needles and syringes placed in puncture-resistant containers	/	Yes, and decontaminated	Yes, and decontaminated	Yes, and decontaminated
Signs				
Biohazard sign if special provisions (e.g. vaccination) required for entry	/	Yes	Yes	Yes
Protective clothing				
Complete change of street clothing to laboratory clothing	/	No, but laboratory coats and gloves required	Yes, special care to minimize skin contamination	Yes, entry/exit only through change and shower rooms
Decontamination of clothing	/	/	Yes	Yes
Ventilated positive pressure Suit	/	/	/	If appropriate
Respiratory protection	/	/	Yes	Yes
Records				
Records of animal use and disposal	/	/	Yes	Yes
Records of incidents and accidents	/	Yes	Yes	Yes
Record of baseline serum samples	/	Yes, If appropriate	Yes, If appropriate	Yes
Record of personnel entry/exit	/	/	/	Yes
Transfer of materials				
Decontamination of material before removal	/	Yes	Yes	Yes, by autoclaving or gaseous/vapour methods
Material container for transport	/	Primary and secondary container required	Primary and secondary container required	Primary and secondary container required
Entry of materials and supplies	/	/	/	Through double-door autoclave or airlock
Others				
Mark all GM neonates within 72 hours after birth	Yes	Yes	Yes	Yes
Eating, drinking, smoking and applying cosmetics not permitted	/	Yes	Yes	Yes
Hand wash before exiting containment area	/	Yes	Yes; or showering	Showering required
Concurrent conduct of experiments with a lower BL	Yes	Yes	Yes	Yes
Animal areas cleaned daily	/	/	Yes	Yes
Minimize creation of aerosols	/	/	Yes	Yes
Separate male and female animals	Yes	Yes	Yes	Yes
Life support system for entilated suits with alarms and backup air tanks	/	/	/	Yes, if such suits are required
Specifications for needles and syringes	/	Yes	Yes	Yes
Quarantine, isolation and edical care facility for personnel	/	/	/	Yes
Preparation and adoption of a biosafety manual	/	Yes	Yes	Yes
Vacuum lines protected with HEPA filters	/	/	Yes	Yes
Appropriate steps to prevent horizontal transmission	/	Yes	Yes	Yes

Annex 6: Facility inspection check list

Different biosafety parameters for containment research are evaluated. They are considered in six different categories.

(i) Laboratory Location and Access

Laboratory Location and Access	Status		Remarks
	Present	Not Present	
Separated from public areas by door.			
Access limited to authorized personnel.			
Laboratory room doors to have appropriate signage (e.g., biohazard sign, containment level, contact information, entry requirements).			
Enough size of door openings to allow passage of all anticipated equipment.			
Doors to the containment laboratory lockable (this does not apply to areas within the containment laboratory).			
Doors to provide restricted access by installation of a controlled access system (e.g., card key) or equivalent.			
Electronic locking systems to be backed up with a physical key-lock system.			
Office areas to be located outside of containment laboratory. Paperwork stations for data collection can be within containment laboratory provided they are located away from laboratory work areas.			
Entry to laboratory to be provided via a waiting room.			
Waiting room door(s) located between the clean and dirty change rooms not to be opened simultaneously with either the containment laboratory door or the clean change entry door. (Interlock, visual or audible alarms, or protocols are all acceptable means.)			
Waiting room door(s) located between the clean and dirty change rooms not to be opened simultaneously with either the containment laboratory door or the clean change entry door (interlock only).			
Interlocked doors, if present, to have manual overrides for emergency exit.			
Entry to laboratory zone to be provided with clothing change areas separating personal and laboratory clothing			

dedicated to that zone (i.e., "clean" change area separated from "dirty" change area).			
Exit from laboratory to be provided with a walk-through shower on the containment barrier (i.e., between “dirty” and "clean" change waiting rooms). (CL3 laboratories manipulating organisms, such as HIV, that are not infectious via inhalation, are not required to fulfill this criterion.)			
Entry to laboratory to be provided via waiting room with airtight doors (e.g., inflatable or compression seal); for laboratories using only a Class III BSC biological safety cabinet line, airtight doors are not required.			
Entry to laboratory zone to be provided with a suit change area, a chemical shower on the containment barrier (i.e., between the laboratory and suit change area) and water shower on exit from the zone (i.e., between "dirty" and "clean" change areas); for laboratories using only a Class III biological safety cabinet line, suit change area and chemical shower are not required.			
Containment laboratories to be located in close proximity to supporting mechanical services to limit the amount of potentially contaminated services.			
Containment laboratories to be located away from external building envelope walls.			
A laboratory support area to be provided adjacent to the containment facility for all supporting laboratory manipulations.			

(ii) Surface (i.e., floors, walls, ceilings, sealants) Finishes and Casework

Surface (i.e., floors, walls, ceilings, sealants) Finishes and Casework	**Status**		Remarks
	Present	**Not Present**	
Doors, frames, casework and bench tops to be non-absorptive (i.e., the use of organic materials should be avoided).			
Working surfaces of bench tops to be non-absorptive			
Surfaces to be scratch, stain, moisture, chemical and heat resistant in accordance with laboratory function.			
Surfaces to provide impact resistance in accordance with laboratory function.			
Surfaces to be continuous and compatible with adjacent and overlapping materials (i.e., to maintain adhesion and a continuous perimeter); wall and floor welded seams are acceptable in level 3 laboratories.			
Continuity of seal to be maintained between the floor and wall (a continuous cove floor finish up the wall is recommended).			
Interior surfaces to minimize movement of gases and liquid through perimeter membrane.			
Interior coatings to be gas and chemical resistant in accordance with laboratory function.			
Interior coatings to be cleanable.			
Structural stability to withstand 1.25 times maximum design pressure under supply and exhaust fan failure conditions (i.e., no wall distortion or damage).			
Bench tops to have no open seams.			
Benches, doors, drawers, door handles, etc. to have rounded rims and corners.			
Reagent shelving to be equipped with lip edges.			

(iii) Heating, Ventilation and Air Conditioning (HVAC)

HVAC	Status		Remarks
	Present	Not Present	
About 100% outside air to be supplied.			
Directional inward airflow provided such that air will always flow towards areas of higher containment.			
Visual pressure differential monitoring devices to be provided at entry to containment laboratory.			
Room pressure differential monitoring lines penetrating the containment barrier to be provided with filters of efficiency filtration.			
Alarm (visual or audible) to be provided in the laboratory and outside laboratory area(i.e., to warn others and maintenance personnel) to signal air handling systems failure.			
Where determined necessary by a local risk assessment, supply air duct to be provided with back-draft protection			
Supply air to be filtered.			
Supply air system to be independent of other laboratory areas.			
Supply air system to be interlocked (i.e., fans, dampers, electrical) with exhaust air system, to prevent sustained laboratory positive pressurization.			
Exhaust air to be filtered (laboratories manipulating organisms, such as HIV, that are not infectious via inhalation are not required to fulfill this criterion.)			
Exhaust air to be passed through two stages of filtration.			
Filters installed into the supply and exhaust system to conform to the standard requirements.			

(iv) Containment Perimeter

Containment Perimeter	Status		Remarks
	Present	Not Present	
Autoclave or other acceptable means of waste treatment/disposal to be provided.			
Double-door barrier autoclave with bioseal to be located on containment barrier; body of autoclave to be preferably located outside of containment for ease of maintenance.			
Barrier autoclave to be equipped with interlocking doors, or visual or audible alarms to prevent both doors from opening at the same time.			
Barrier autoclave to be equipped with interlocking doors, and visual or audible alarms to prevent both doors from opening at the same time.			
For materials that cannot be autoclaved (e.g., heat sensitive equipment, samples, film) other proven technologies for waste treatment (e.g., incineration, chemical, or gas) to be provided at containment barrier.			
All penetrations to be sealed with non-shrinking sealant at containment barrier.			
All conduit and wiring to be sealed with non-shrinking sealant at the containment barrier.			
Windows, if they can be opened, to be protected by fly and pollen screens.			
Windows positioned on containment barrier to be sealed in place; window glazing material to provide required level of security.			
Observation windows to be installed on containment barrier.			

(V) Laboratory Services (i.e., water, drains, gas, electricity, and safety equipment)

Laboratory Services (i.e., water, drains, gas, electricity, and safety equipment)	Status		Remarks
	Present	Not Present	
Hooks to be provided for laboratory coats at laboratory exit; street and laboratory clothing areas to be separated.			
Hand-washing sinks to be located near the point of exit from the laboratory or in waiting room.			
Hand-washing sinks to be provided with "hands-free" capability.			
Other primary containment devices to be provided.			
Emergency eyewash facilities to be provided in accordance with applicable regulations			
Emergency shower equipment to be provided in accordance with applicable regulations			
Domestic water branch piping serving laboratory area(s) to be provided with backflow prevention, and isolation valve, to be located in close proximity to the containment barrier.			
Drain lines and associated piping (including autoclave condensate) to be separated from lower containment laboratory areas and to go directly to main building sanitary sewer at point of exit from building (downstream of all other connections).			
Drain lines and associated piping (including autoclave condensate) to be separated from areas of lower containment and to be connected to an effluent sterilization system.			
Drains connected to effluent sterilization to be sloped towards sterilization system to ensure gravity flow; consideration should be given to the installation of valves to isolate sections of piping for *in situ* decontamination; the effluent sterilization system (e.g., piping, valves, tank) to be heat and chemical resistant consistent with application.			
Autoclave condensate drains to have a closed			

connection.			
Drainage traps to be provided to required deep seal depth in consideration of air pressure differentials.			
Floor drains not to be provided, except when essential (e.g., body shower and animal rooms).			
Plumbing vent lines (including effluent sterilization system) to be provided with filter of efficiency and provided with a means of isolation and decontamination.			
Plumbing vent lines to be independent of lower containment plumbing vent lines, or combined with lines from lower containment when provided with a filter of efficiency.			
Compressed gas cylinder(s) to be located outside the laboratory.			
Laboratory supply gas piping (e.g., carbon dioxide, compressed air) to be provided with backflow prevention.			
Portable vacuum pump to be provided in the laboratory. Internal contamination of vacuum pump to be minimized			
Compressed breathing air to be provided to positive-pressure personal protective equipment (i.e., for connection to the air hose of suits), equipped with breathing air compressors and back-up cylinders (sufficient for 30 minutes per person); air hose connections to be provided in all areas where suits are worn, including chemical shower and suit change room.			
Emergency lighting to be provided.			
Life safety systems, lighting, security systems and other essential equipment to be supported with emergency back-up power.			
Circuit breakers to be located outside biocontainment area.			
Laboratory to be equipped with a communication system between containment area and outside support area.			

System (e.g., fax, computer) to be provided for electronic transfer of information and data from laboratory area to outside laboratory perimeter. (Note: paperwork from the decontamination, i.e., following autoclaving, irradiation, containment laboratory may be removed after appropriate microwaving; such practices are generally not recommended for use on a routine basis).			
Work area to be monitored (e.g., closed circuit TV) from outside laboratory perimeter (e.g., security/biosafety office).			
Fire extinguisher			
First Aid Kit			

(vi) Storage Facility

Storage facility	status		Remarks
	Present	Not present	
The store is clean, sanitized, intact and labeled			
Containers are sealed and labeled			
Multiple samples of one transgenic materials be stored separately in sealed containers			
Multiple samples of different transgenic materials stored separately in sealed containers			
Forms for storage inspection are available and recorded accordingly			
Containment of accidentally released transgenic materials is effected			
Protocols for notification of accidental release of transgenic materials to relevant parties are filled in the record of corrective action			
Record of storage inspection retained in compliance document binder			
Doors to the store are lockable			
Store windows are closed and locked			
Store be sanitized immediately after use			
Disposed transgenic materials destroyed by dry heat/stem heat/ crushing/ burning/ autoclaving/ chemical treatment			
Compliance document binder available to TPRI inspectors upon request			

Additional Remarks.

.....

Inspected by:

Name of Plant Biosafety Inspector:……

Signature ……. Date……

Annex 7: Application form for Contained GM Research in Tanzania

FORM A: Application Form for Contained GM Research in Tanzania

UNITED REPUBLIC OF TANZANIA
VICE PRESIDENT'S OFFICE
DIVISION OF ENVIRONMENT

Γ **CBI COPY**

Γ **NO CBI**

General Instructions

This application form consists of seven parts which must be completed for any type of research involving **genetic modification** under containment in Tanzania.

All sections of this application must be completed. If the space provided is not sufficient, attach additional supporting materials as necessary. Page numbering and headings of any supplementary material must match corresponding sections in this application.

Submit 5 copies of the application for use by the NBFP in both hard and soft forms by regular mail or courier delivery.

If completion of this application requires the disclosure of confidential business information (CBI), then both CBI and non-CBI copies of the application must be submitted.

Provide additional hard copy of the application containing no confidential information. The latter application will be made available for public scrutiny.

Conduct a public notification in accordance to biosafety regulations of Tanzania.

The appropriate fee as stipulated in the biosafety regulations must accompany the application. Please note that the Vice president's office does not accept cash.

Applications must be received by national Biosafety Focal Pont (NBFP) at the address shown below at least **180 days in advance** of the commencement date of the proposed research.

Permanent Secretary
Vice President's Office
P.O. Box 5380
Dar es Salaam
Tanzania
Email: info@vpodoe.go.tz
Fax +255 22 2125297
Tel: +255 22 2113983/2118416

PART 1: APPLICANT/ADMINISTRATIVE INFORMATION

1.1 Contact Details of Principal Investigator (PI)

Name of Principal Investigator (PI) Postal address: Physical Address: Telephone number: Cell phone number: Fax: E-mail: Attach: Current CV Name of and Contact of three referees

1.2 Name and address of the institution

Name of the institution Postal address: Physical Address: Telephone number: Cell phone number: Fax: E-mail: Website**:**

1.3 Institutional Biosafety Committee (IBC)

Name of the IBC **Chairperson:** **Secretary:** Postal address: Physical Address: Telephone number: Cell phone number: Fax: E-mail

1.4 Title and purpose of the application

Title: Purpose:

1. 5 Previous application or approval

Details of previous application or approval:

PART 2: INFORMATION ABOUT THE PROJECT

2.1 Title of the project

2.2 Proposed date of commencement of the project

2.3 Proposed date of completion of the project

2.4 Brief description of the project

PART 3: DESCRIPTION OF THE GMO

3.1 Common and scientific names of the parent organism

Common name Common and scientific name(s)

3.2 Vector(s) or methods to be used for the transfer of genetic material

Indicate method of transformation; promoter, selection marker to be used

3.3 Class of the modified trait

(E,g. Pest resistance, drought tolerance, Disease resistance, Biofortification et.)

3.4 Modified trait

(E,g. Resistance to Bollworm, elevated Provitamin A etc.)

3.5 Identity and function of the gene(s) responsible for the modified trait

E.g. resistance to bollworm, elevated provitamin A etc

3.6 Organism (s) from which the gene(s) responsible for the modified trait(s) were isolated

3.7 Organisms or tissues to be used in association with the GMO

PART 4: ADDITIONAL INFORMATION FOR A GMO THAT IS A WHOLE PLANT OR IS TO BE USED IN CONJUNCTION WITH A WHOLE PLANT

4.1 Stage of plant development to be grown

4.2 Growing medium for the plants

PART 5: RISK ASSESSMENT AND MANAGEMENT

Health and safety of people, animals and environment

5.1 Biosafety Risk Level

Indicate the biosafety risk level

5.2 Pathogenicity risks of the vector or construct

Indicate infectious dose, mode of transmission, host range, availability of preventive measures and availability of effective treatment

5.3 Possibility of aerosol generation- eg pollen etc

5.4 Possible hazard(s) and the likelihood and consequence of the hazard(s) occurring (*i.e.* the risk) from the proposed genetic modification(s)

5.5 Possible hazard(s) and the likelihood and consequence of the hazard(s) occurring (*i.e.* the risk) from an unintentional release of the GMO(s) into the environment?

5.6 Related incident and emergency response

5.7 Physical requirements

Indicate the physical infrastructure/facilities required for safe conduct of your dealing

5.8 Operational Requirements

Indicate operational requirement for safe conduct of your dealing

5.9 Transportation of the GMOs outside the contained facility

Indicate if the transformed materials will be transported outside the contained facility, and if so how

5.10 Storage of the GMO

5.11 Personnel suitability and reliability

5.12 Liability and Accountability

Indicate the name and designation of person

5.13 Disposal of the GMOs

Describe how the transformed materials will be disposed

5.14 Other actions and precautions to be taken to minimise risks posed by the proposed dealing(s)

Describe other safety measures that will be put in place to minimize any potential risks

PART 6. DESCRIPTION OF THE CONTAINMENT FACILITY

6.1 Information of the facilities to be used

Give brief description of the containment facility and provide sketch Facility type Physical containment level: Address:

6.2 Facility contact person details

Name: Business phone number: Mobile phone number: Facsimile number: E-mail address:

PART 7: DECLARATION AND SIGNATURES

I hereby declare and certify that the information in this application is complete and accurate to the best of my knowledge and belief.

7.1 Principal investigator and Project supervisor

Principal Investigator of the Applying Institution	
Name:	
Signature**:**	Date:
Project Supervisor	
Name:	
Signature	Date:

7.2 Affidavit/ Compliance Agreement

Complete the affidavit/ compliance agreement *Note: The affidavit/ compliance agreement is an inseparable part of the application form*

Annex 7, 8, 9,10: SOP for operational /management issues

Annex 11 Microorganisms

REFERENCES

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