

THE SINGAPORE BIOSAFETY GUIDELINES FOR RESEARCH ON GENETICALLY MODIFIED ORGANISMS (GMOs)



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SECTION 1: INTRODUCTION

1.1 BACKGROUND

GMAC was set up in April 1999 to oversee and advise on the issues relating to genetic modification (GM) and genetically modified organisms (GMOs).

GMAC's primary objective is to ensure public and environmental safety, while allowing for the commercial use of GMO and GMO-derived products by companies and research institutions, in compliance with international standards.

These guidelines were drawn up after a review of relevant guidelines, regulations and publications including those from Australia, United States of America (USA), Europe, World Health Organization (WHO) and United Nations Environment Programme (UNEP). A list of all relevant documents and references are listed in Appendix 1.

All communications with GMAC should be addressed to the Secretariat.

Secretariat, Genetic Modification Advisory Committee (GMAC)

20 Biopolis Way # 08-01 Centros, Singapore 138668

Email : info@gmac.gov.sg

Website : <http://www.gmac.gov.sg>

1.2 OBJECTIVES OF GUIDELINES

These guidelines are established to ensure the safe containment, handling and transport of genetically modified organisms used in research and to provide a common framework for assessment and notification of research on GMOs.

1.3 SCOPE OF GUIDELINES

The scope of the Singapore Biosafety Guidelines for Research on GMOs covers experiments that involve the construction and/or propagation of all biological entities (cells, organisms, prions, viroids or viruses) which have been made by genetic manipulation and are of a novel genotype and which are unlikely to occur naturally or which could cause public health or environmental hazards.

1.4 DEFINITIONS OF ABBREVIATIONS

For purposes of these Guidelines, the following abbreviations shall be defined as follows.

- “GMOs” refers to Genetically Modified Organisms
- “GMMs” refers to Genetically Modified Microorganisms
- “GMAC” refers to the Genetic Modification Advisory Committee of Singapore.
- “AVA” refers to the Agri-Food and Veterinary Authority of Singapore
- “MOH” refers to the Ministry of Health, Singapore
- “NEA” refers to the National Environment Agency, Singapore
- “MOM” refers to the Ministry of Manpower, Singapore
- “BATA” refers to the Biological Agents and Toxins Act 2005 (Act 36 of 2005), regulated by the Ministry of Health
- “IBC” refers to the Institutional Biosafety Committee
- “NACLAR” refers to the National Advisory Committee for Laboratory Animal Research
- “BAC” refers to the Bioethics Advisory Committee

A detailed glossary of terms can be found in Appendix 21.

SECTION 2: PURVIEW - EXTENT AND EXEMPTIONS

2.1 EXTENT OF GUIDELINES

- 2.1.1 **These guidelines cover experiments that involve the construction and/or propagation of all biological entities (cells, prions, viroids, viruses or organisms) which have been made by genetic manipulation and are of a novel genotype¹ and which are unlikely to occur naturally, or which could cause public health or environmental hazards. These guidelines do not cover work involving human subjects.**

The categories of experiments which fall under these guidelines are described in Section 3. A list of other relevant documents is included in Appendix1.

- 2.1.2 Intentional release of genetically manipulated organisms must adhere to the "Singapore Guidelines on the Release of Agriculture-related Genetically Modified Organisms" (GMAC).
- 2.1.3 If an investigator is unsure whether their research proposal falls within these guidelines, a description of their proposed research should be submitted, in writing, to their Institutional Biosafety Committee(s) (IBC) for clarification, before the commencement of their research work.

The responsibilities of GMAC, IBCs and principal investigators (laboratory leaders) are defined in Section 5.

¹ In the case of multicellular organisms, 'novel genotype' refers to any of the cells which make up the organism.

2.2 EXEMPTIONS

2.2.1 The following classes of experiments ((i) - (vii)) are exempt from the guidelines unless they fall within Categories A or B in Section 4. Such experiments exempt from the guidelines are classified as Category C (refer also to section 4.3).

- (i) Experiments involving the fusion of mammalian cells which generate a non-viable organism, for example, the construction of hybridomas to make monoclonal antibodies.
- (ii) Fusion of protoplasts between non-pathogenic micro-organisms.
- (iii) Protoplast fusion, embryo-rescue, *in vitro* fertilisation or zygote implantation in plant cells.
- (iv) Experiments involving the breeding or use of genetically modified model organisms, namely mice, rats, zebrafish, *Xenopus* spp. and *Arabidopsis* spp. in which:
 - (a) the genetic modification involves knock-out, deletion and inactivation of genes; and/or
 - (b) the genetic modification using knock-in, activation, gene substitution and activation of genes in which the outcome does not confer any survival advantage to the animal and cause subsequent detrimental effects in human health and environment through the expression of toxins and/or human pathogens. These may include selectable reporter/marker genes such as fluorescent proteins.

If further manipulations are performed on any of the above described transgenic organisms, they may not be automatically exempted from the guidelines and could fall within Category B(i) (Risk Group 2) or Category A as assessed separately.

- (v) Research involving the introduction of naked nucleic acids into plants or animals (other than humans), unless the nucleic acid is both recombinant and able to give rise to infectious agents.
- (vi) Work involving the introduction of genetically manipulated somatic cells into animals, unless they are able to give rise to infectious agents.
- (vii) Experiments involving approved host/vector systems (refer to Appendix 2) provided that the donor DNA:
 - is not derived from plant or animal pathogens and that the DNA to be introduced is characterised fully and will not increase host or vector virulence;
 - is derived from mammalian sources and is used to construct shot-gun libraries in an approved host/vector system mentioned in Appendix 2;

- does not code for a vertebrate toxin having a LD50 of less than 100 µg/kg;
- does not represent or comprise more than two-thirds of a viral genome, and is not being used in any experiment in which missing segments of the viral genome that are essential for infection are available in the host cell or will become available by further breeding processes.

2.2.2 All experiments, whether exempt or not, should be carried out under conditions of standard microbiological laboratory practice. When pathogenic organisms are used, appropriate containment levels should be used and the personnel should be properly trained and have had the recommended vaccinations as stipulated in the guidelines issued or recommended by regulatory authorities such as MOH and AVA, e.g. Laboratory Biosafety Manual, 3rd Ed, World Health Organization.

2.2.3 Exemptions under Sections 2.2.1 (i) – 2.2.1 (vii) **do not apply** should the experiments involve intentional releases of genetically manipulated organisms, which include contained field trials. Such experiments must adhere to the Singapore Guidelines on the Release of Agriculture-related Genetically Modified Organisms.

2.2.4 Exemption from these guidelines does not equal exemption from statutory provisions applying to any aspect of a project involving genetic manipulation (e.g. importation, quarantine legislation).

2.3 WORK SUBJECTED TO OTHER AND/OR ADDITIONAL REGULATORY REQUIREMENTS

2.3.1 Work with GMOs derived from biological agents and toxins known to be hazardous to human health are regulated, under the Biological Agents and Toxins Act (Act 36 of 2005). Large scale production of GMOs derived from biological agents and toxins known to be hazardous to human health may be regulated under the Biological Agents and Toxins Act (2005). Large scale production refers to the production of 10 or more litres of biological agent at any one time.

2.3.2 Work in the field of human health such as gene therapy, or other genetic manipulations on humans involving stem cells, whole organs or individuals will be assessed by its designated agency(ies). These investigations include the introduction of nucleic acids (genetically manipulated or chemically synthesised and their derivatives), or genetically manipulated micro-organisms, or cells into human subjects for the purposes of gene therapy, cell marking, or for stimulating an immune response against a subject's own cells, as used for the treatment of some cancers.

2.3.3 Research proposals where the introduction into human subjects of nucleic acids (genetically manipulated or chemically synthesised), or genetically manipulated micro-organisms, or cells, is designed to stimulate an immune response to antigenic determinants of infectious agents, as in the case of classical vaccine, should be submitted to the appropriate Bioethics committees. If necessary, advice from GMAC could also be obtained.

- 2.3.4 Vaccines which have been approved for use in Singapore, as well as the transfer of non-genetically manipulated autologous host cells, organ and tissue transplants are subjected to other relevant authority requirements. If necessary, advice from GMAC could also be obtained.

2.4 REGULATORY AGENCIES

The national agencies responsible for legislating the various aspects of GM technology and activities pertaining to GM technology and GMOs in Singapore are:

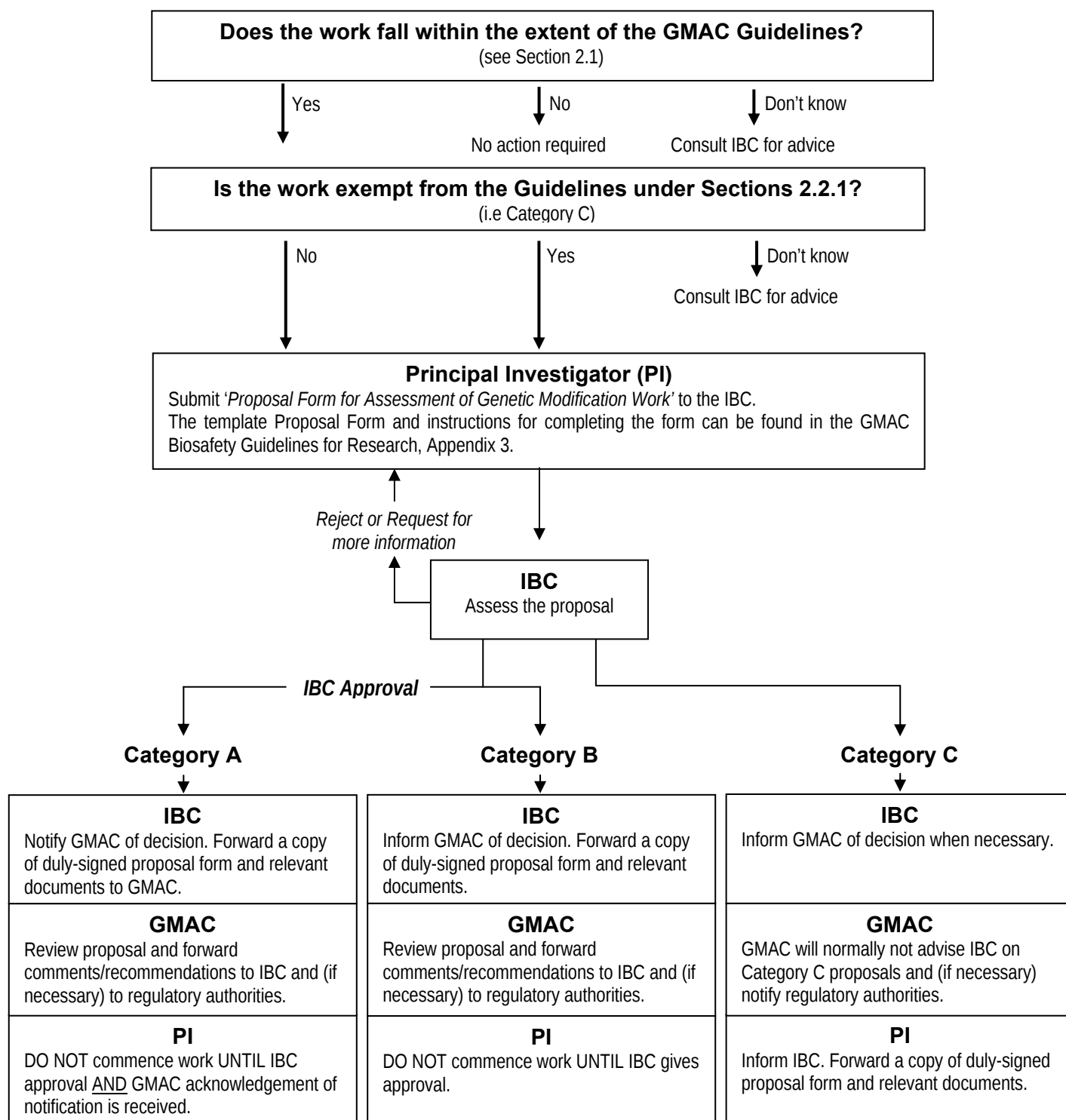
- Regulation of laboratories dealing with GMO research, involving animal pathogens and plant pests – AVA
- Importation of organisms including GMOs – AVA, MOH and NEA (please also see Section 6)
- Certification or Inspection of Laboratories handling biological agents or toxins regulated under the BATA – MOH
- Regulation of Workplace Safety and Health - MOM

The requirements of the regulatory bodies should be met; see Appendix 1 for the relevant local Acts.

SECTION 3: SUMMARY OF PROCEDURES

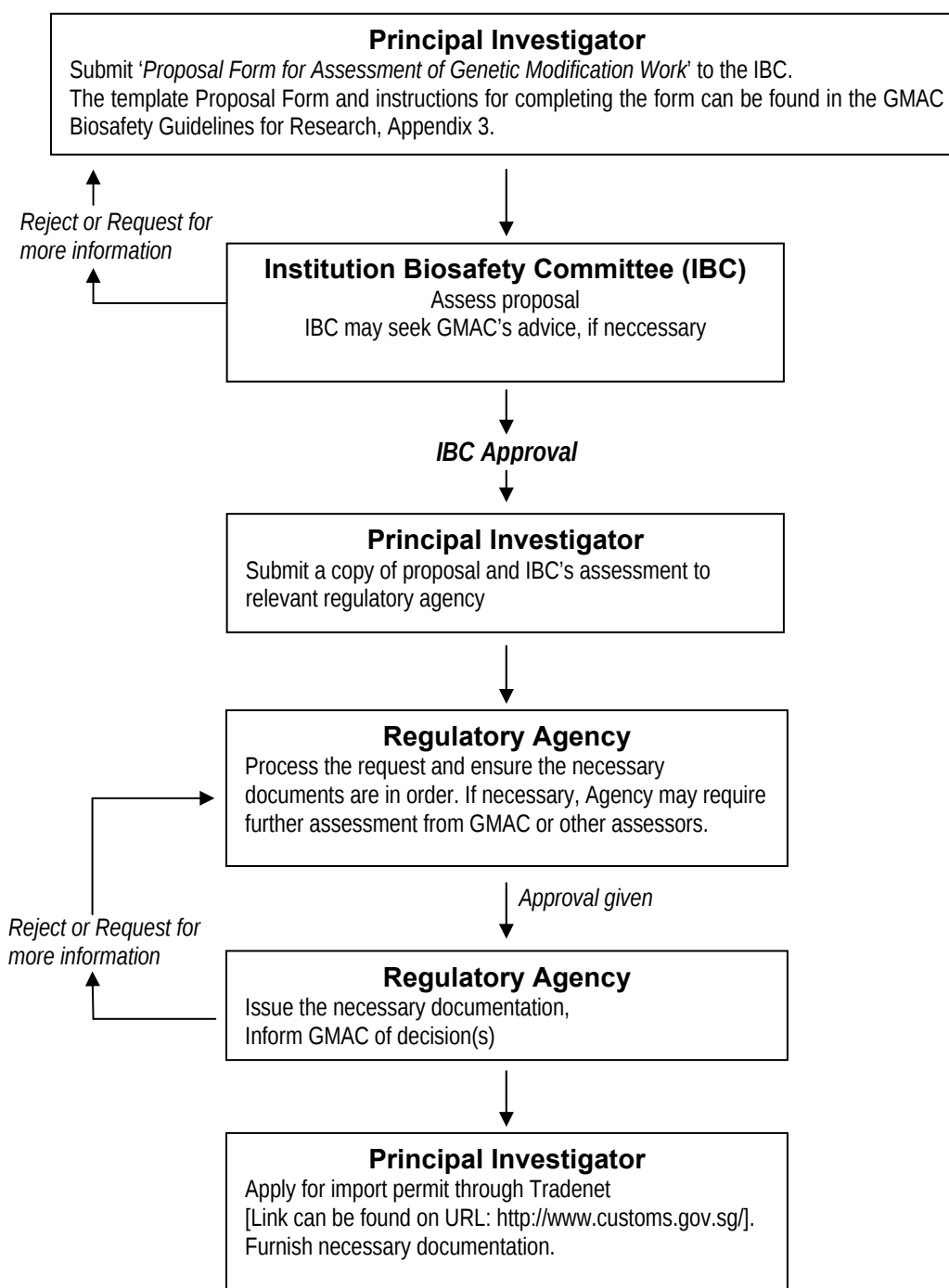
3.1 DECISION FLOW CHART FOR ASSESSMENT AND NOTIFICATION OF RESEARCH WORK

The relevant procedures for assessment and notification of research work involving genetic manipulation are detailed in Sections 3.3 – 3.5. A simplified Decision Chart is shown below for easy referral.



3.2 FLOW CHART FOR IMPORTATION OF GMOs FOR RESEARCH (Applicable to GMOs falling under Category A and/or B)

Note that importation or possession of GMOs, not relating to human health, is regulated under Section 9 of the Animals and Birds Act (Cap 7). Additionally, importation or procurement to import specific biological agents and toxins, which is capable of causing death, disease or malfunction in a human, as those specified under First, Second, Fourth and Fifth Schedules, is regulated under the Biological Agents and Toxins Act (Act 36 of 2005).



3.3 **CATEGORY A – EXPERIMENTS REQUIRING IBC APPROVAL AND GMAC NOTIFICATION (REGULATED EXPERIMENTS WITH SIGNIFICANT RISKS)**

Please refer to Section 4.1 for a description of experiments falling into Category A.

Experiments in this category **require both IBC assessment and GMAC notification** prior to commencement. Principal investigators should **not commence work** on proposals assessed as Category A until advised by their IBC, which will not transpire until the IBC receives acknowledgement of GMAC notification.

Principal Investigators intending to conduct experiments classified as Category A should submit a 'Proposal Form for Assessment of Genetic Manipulation Work' to their respective IBCs for assessment. The IBC shall assess the proposal and determine the appropriate working and containment measures and facilities necessary.

A proposal in this category should be submitted by the Principal Investigator to the IBC for assessment on a GMAC 'Proposal Form for Assessment of Genetic Manipulation Work'. The IBC should assess the proposal and determine the appropriate working and containment conditions. Upon approval, the IBC should forward the proposal to the GMAC secretariat, together with a summary of the IBC's recommendations or comments, for notification.

Instructions for filling out the forms are included in Appendix 3. When completing the forms and assessing the experiments, the IBC and the investigator should identify potential hazards and their types, and decide upon any special procedures necessary for the proposed experiments.

The GMAC Secretariat will return a copy of the GMAC 'Proposal Form for Assessment of Genetic Manipulation Work', carrying a GMAC case reference number, to the IBC within 10 working days to acknowledge receipt of the proposal. The case reference number should be quoted in all future correspondences relating to the proposal. The acknowledgment of receipt does not imply GMAC's acknowledgement or endorsement of IBC's decision. Work must not commence until GMAC has released its acknowledgement and/or endorsement to the IBC.

3.4 **CATEGORY B – EXPERIMENTS REQUIRING IBC APPROVAL (NOTIFIABLE EXPERIMENTS WITH LOW RISKS)**

Please refer to Section 4.2 for a description of experiments falling into Category B.

Experiments in this category **require assessment by the IBC** prior to commencement. Principal investigators **should not commence Category B experiments** until advised by the IBC.

Principal Investigators intending to conduct experiments classified as Category B should submit a 'Proposal Form for Assessment of Genetic Manipulation Work' to their respective IBCs for assessment. The IBC shall assess the proposal and determine the appropriate working and containment measures and facilities necessary.

The IBC shall forward a copy of the approved proposal form, together with a summary of the IBC's recommendations, to the GMAC Secretariat. GMAC will review and forward comments/recommendations to IBC and if necessary, to relevant regulatory authorities.

The GMAC Secretariat will return a copy of the GMAC 'Proposal Form for Assessment of Genetic Manipulation Work', carrying a GMAC case reference number, to the IBC within 10 working days to acknowledge receipt of the proposal. The case reference number should be quoted in all future correspondences relating to the proposal. The acknowledgment of receipt does not imply GMAC's acknowledgement or endorsement of IBC's decision. Principal Investigators can commence work concurrently with GMAC notification.

3.5 CATEGORY C – EXPERIMENTS EXEMPT FROM THE GUIDELINES (EXPERIMENTS WITH NO SIGNIFICANT RISKS)

Please refer to Section 4.3 for a description of experiments falling into Category C.

Experiments in this category are exempt from the guidelines and therefore do not require GMAC notification.

Principal investigators who are unsure if their work falls under the exemptions in Sections 2.2.1 (i) – 2.2.1 (vii) should submit a proposal on a 'Proposal Form for Assessment of Genetic Manipulation Work' to their respective IBCs for assessment. The IBC shall assess the proposal and determine the appropriate categorization. Principal Investigators are encouraged to inform IBC of their decision by forwarding a duly-signed proposal form and relevant documents. GMAC will normally not advise the IBC on Category C proposals, unless necessary.

SECTION 4: EXPERIMENTS COVERED BY THE GUIDELINES

4.1 CATEGORY A – EXPERIMENTS REQUIRING IBC APPROVAL AND GMAC NOTIFICATION (REGULATED EXPERIMENTS WITH SIGNIFICANT RISKS)

This category includes experiments which may pose high risks to laboratory workers, the community or the environment. This category also includes experiments for which the type or level of hazard is unclear. The level of containment required will vary depending on the kind of experiments and their assessed hazard.

In general, experiments involving biological agents or toxins that are defined as First, Second and Fifth Schedules of the BATA and/or classified as Risk Group 3 and 4 of the WHO Laboratory Biosafety Manual will fall within this Category A.

A facility of containment of, at least, level BSL2 (as determined by the IBC and with reference to the Biosafety level detailed by the ATCC and CDC) is required.

This category of work **requires IBC assessment and approval, followed by GMAC notification before work begins**. Principal investigators **should not commence work** on proposals assessed as Category A until advised by the IBC, following IBC's receipt of GMAC acknowledgement of notification. Please refer to Section 3 for procedures for submitting proposal forms and obtaining GMAC advice.

The following classes of experiments fall within Category A:

A(i) Experiments with toxin producers:

- Experiments using DNA which encodes a vertebrate toxin having an LD50 of less than 100 µg/kg. Appendix 4 lists some toxins falling under this sub-category.
- Experiments in which toxin genes are expressed at a high-level, even if the LD50 is greater than 100 µg/kg. Experiments using uncharacterised DNA from toxin-producing organisms and, which therefore, could contain toxin sequences also falls under this sub-category. However, experiments using DNA which has been fully characterised and shown not to code for a toxin, from a toxin-producing organism as donor, is not included in this sub-category.

A(ii) Experiments using viral vectors whose host range includes human cells, and where the viral vectors contain one or more inserted DNA sequences coding for a product known to play a role in the regulation of cell growth or to be toxic to human cells. (Special conditions for working with viral vectors encoding oncogenes are given in Appendices 5 and 6.)

A(iii) Experiments involving introduction of DNA into microorganisms which can cause plant or animal (including human) diseases when used as host or vector, except:

- (a) microorganisms listed as approved hosts or vectors (see Appendix 2); or
- (b) if the DNA will not increase the virulence of the host or vector and the DNA is fully characterised, in which case it is classified as B(iii).

This sub-category does not include experiments using replication-defective viruses as host or vector. However, experiments using defective vector/helper virus combinations which have the potential to regenerate non-defective recombinant virus are included in this sub-category.

- A(iv) Introduction of pathogenicity genes into microorganisms other than the approved hosts included in Appendix 2. This sub-category includes those genes whose products are suspected of, or have a risk of initiating autoimmune diseases.
- A(v) Cloning or transfer of entire viral genomes, viroids, or fragments of a genome capable of giving rise to infectious particles with the capacity to infect human, animal or plant cells. Experiments involving cloning of less than two-thirds of an entire viral genome do not fall within this sub-category. Cloning of a viral genome which lacks a vital component of its replication or packaging activity that is not supplied by the experimental system, also does not fall within this sub-category.
- A(vi) Experiments involving recombination between entire viral genomes, viroids and/or complementary fragments of these genomes, where one or more fragments encode virulence or pathogenic determinants. This sub-category includes experiments that could alter the host range of pathogens or increase pathogen virulence or infectivity.
- A(vii) Experiments where a fragment of or the entire genome of a virus is injected into an embryo to produce a transgenic animal which secretes or produces infectious viral particles (see Appendix 12)
- A(viii) Experiments not falling within the A sub-categories listed above or into Category B, but which fall within the extent of the guidelines (see Section 2).

4.2 CATEGORY B – EXPERIMENTS REQUIRING IBC APPROVAL (NOTIFIABLE EXPERIMENTS WITH LOW RISKS)

This category includes experiments which may pose low-level risks to laboratory workers, the community or the environment. These experiments require at least Biosafety Level 2 physical containment (laboratory, plant house, animal house, insectary, bird house or aquarium), as determined by the IBC and with reference to the Biosafety level detailed by the ATCC and CDC. Some experiments may require additional precautions or higher containment because the donor DNA or its components are hazardous or infectious, for example special containment features are needed for the housing of transgenic animals. Recommendations for procedures for GM-BSL2 and other containment levels are in the appendices (Appendix 7 *et seq*).

IBC assessment is required before work begins on this category of experiments. Principal investigators **should not commence work** on proposals assessed as Category B until specifically advised by the IBC. Procedures for submitting proposal forms and obtaining IBC advice are in Section 2.

If the proposed experiments should fall within both Categories A and B, Category A classification shall take precedence i.e. Category A conditions shall be applied.

The following classes of experiments fall within Category B:

- B(i) Experiments with whole animals (including non-vertebrates) which involve genetic manipulation of oocytes, zygotes or early embryos to produce a novel organism. For transgenic animal work, prior approval from the institution's bioethics committee is needed. (See Appendix 1 for other relevant documents, Appendix 12 on the relevant administrative procedures required by these Guidelines, and Appendix 13 *et seq* for transgenic animal facility requirements.)
- B(ii) Genetic manipulation experiments involving the production of modified whole plants.
- B(iii) Work with non-approved host/vector systems (i.e. other than those listed in Appendix 2) where the host or vector either:
 - (a) does not usually cause disease in plants, humans or animals; or
 - (b) is able to cause disease in plants, humans or animals but the introduced DNA is completely characterised and will not cause an increase in the virulence of the host or vector.
- B(iv) Experiments with approved host/vector systems, in which the gene inserted is: (a) a pathogenic determinant; or (b) DNA that is not fully characterised from microorganisms which are able to cause disease in humans, animals or plants; or (c) an oncogene. (See Appendices 5 and 6 for work with oncogenes.)

Shot-gun cloning of mammalian DNA in approved host/vector systems does not fall into this category. Approved host/vector systems are listed in Appendix 2. Investigators may request to have new host/vector systems added to the list by making a detailed submission to GMAC through their IBC.

Note that experiments not falling within B sub-categories or in Category A, but falling under the Extent in Section 2, require GMAC advice and IBC approval (see sub-category A(viii)).

4.3 CATEGORY C – EXPERIMENTS EXEMPT FROM THE GUIDELINES (EXPERIMENTS WITH EXTREMELY LOW RISKS)

This category includes experiments which do not pose significant risks to laboratory workers, the community or the environment.

Experiments falling into this category include those classes of experiments as outlined in Section 2.2.1 (i) – 2.2.1 (vii).

Principal Investigators who are unsure of the categorization of their experiments are required to seek advice from their respective IBCs, by submitting a 'Proposal Form for Assessment of Genetic Manipulation Work'. The IBC shall assess the proposal and determine the appropriate categorization status.

SECTION 5: ROLES AND RESPONSIBILITIES

5.1 INSTITUTIONS

Any institution, company or organisation that carries out genetic manipulation, imports organisms arising from such work, produces such organisms, or plans to sell or release such organisms into the environment, should abide by all existing legislation and relevant guidelines, especially current GMAC Guidelines.

Such institutions, companies or organisations are required to establish an IBC and provide the resources and facilities which are necessary for safe work in laboratories. The IBC should carry out its duties adequately, and ensure, by recruitment, procedures and other measures that adequate supervision of work occurs. Institutions may consider making compliance with the GMAC Guidelines a term of their employment contracts, if appropriate. Those Institutions conducting large scale or industrial scale work should have a Biological Safety Officer.

GMAC appreciates the difficulties that small institutions and companies may have in establishing an IBC. These institutions and companies can choose to be supervised by another IBC. Such arrangements should be formalised between the institutions concerned, and the GMAC notified. A representative of the smaller institution should closely liaise with, or be a member of, the IBC.

The responsibilities of IBCs are described in Section 5.2. Institutions should recognise the essential roles of their IBC and give it the authority and support it needs to undertake its duties.

5.1.1 Recruitment and staff training

The institution should ensure that laboratory staff is informed of hazards and have adequate training to make sure that their work is carried out within these guidelines. The IBC Chair or Biological Safety Officer should be readily accessible to give advice.

5.1.2 Certification of Biosafety Level 3 (BSL3) and Biosafety Level 4 (BSL4) facilities

BSL3 and BSL4 facilities (laboratories, animal houses, plant houses, insectaries, bird houses, aquaria) are to be approved by the regulatory agency(ies). Institutions planning to conduct experiments which require BSL3 or BSL4 containment should notify the GMAC Secretariat. The necessary advice regarding the structural requirements about these BSL3 and BSL4 facilities are included in Appendices 8, 9, 11, 15 and 17. These advice may also be found in the Laboratory Biosafety Manual, 3rd Ed, WHO.

Appropriate door signs and universal Biohazard signs and labels are available from laboratory and reagent suppliers.

5.2 INSTITUTIONAL BIOSAFETY COMMITTEES

IBCs are vital for executing these guidelines and thus the monitoring and surveillance of genetic manipulation work. The calibre and experience of IBC members should be such that it can competently undertake its duties. The Chair of the Committee should be of sufficient standing in the institution for decisions and advice by the IBC to be effectively carried out. Appropriate deputising arrangements should be made when the Chair is on leave.

5.2.1 Where applicable, duties and obligations in relation to biological agents and toxins, as stipulated in the Part V of the BATA legislation must be adhered to.

5.2.2 Composition

The IBC should comprise:

- individuals with requisite knowledge and expertise to evaluate and oversee work being conducted in the institution;
- the Biological Safety Officer, if appropriate;

One microbiologist, and one molecular biologist and/or a geneticist, should be included as well as a scientist with expertise relevant to the organisms being studied in the institution. Different disciplines need only be represented when work falling within that area is performed in the institution. For example, an institution working only on plants need not have an animal geneticist.

Responsibilities may be combined in the same person if appropriate.

5.2.3 Biological Safety Officer

Institutions should either appoint a Biological Safety Officer, or assign such duties to the IBC. If institutions have more than one officer, for the purposes of these Guidelines, only one name per institution is to be submitted to GMAC in the annual reporting requirements. The officer should ideally have experience in working with containment conditions and should be sufficiently trained and competent to offer advice on, or participate in staff training. Suitable deputising arrangements should be made when the officer is on leave.

The Biological Safety Officer or the IBC Chair should act as adviser to the head of the institution or company in all biosafety matters. Regular safety audits and the supervision of a regular evaluation program for relevant pieces of equipment should be carried out by the Biological Safety Officer or the IBC. The Biological Safety Officer should also consult the WHO Laboratory Biosafety Manual for additional requirements that need to be fulfilled.

5.2.4 Conflicts of Interests

To avoid any potential conflicts of interest, IBC members should not assess their own proposals that they have submitted. The IBC should have sufficient scientifically qualified members to ensure that proposals can be adequately evaluated.

IBC members who may have commercial interests on an item of the agenda being assessed should declare their interests and be excluded from the meeting.

5.2.5 Monitoring of Work

The IBC should ensure that GMAC's and its own advice on proposals are received by principal investigator(s) and, if necessary, are acted upon. The IBC should visit laboratories and facilities occasionally to monitor biosafety aspects and implementation.

In order to effect the intent of these guidelines, an IBC may draft whatever rules it considers necessary to supplement these guidelines. Furthermore, IBCs should have appropriate powers to ensure that all aspects of these guidelines are observed.

5.2.6 Duties

The main functions of the IBC are to:

- assess all research proposals it receives, (including changes to Category C projects), so as to identify potential hazards to the researchers, the public and the environment. It should also advise the investigator(s) about these hazards and their management;
- ascertain the containment level and procedures for experiments falling within GMAC Categories A and B (see Section 4), and determine the storage and transportation requirements for genetically manipulated organisms falling within these Guidelines;
- send an original typed GMAC 'Proposal Form for Assessment of Genetic Manipulation Work', together with the IBC's assessment, to the GMAC for assessment of experiments falling under Category A, and make sure that GMAC advice is followed. For Category B, an original typed proposal form, together with the IBC's assessment, should be sent to GMAC for notification. See Appendix 3 for instructions on completing proposal forms;
- inspect plant houses, animal houses, bird houses, insectaries and aquaria before they are used for genetic manipulation work. The IBC should also conduct inspections and monitor procedures in **all** the institution's containment facilities. At least annual inspections of these facilities should be carried out to make sure that they continue to meet the relevant containment standards. The detailed requirements for these facilities are in Appendices 7 *et seq*;
- monitor ongoing work within the institution from time to time and make recommendations to investigators, if appropriate;
- assess the qualifications and experience of personnel involved in research proposals, to make sure that they are adequate for good microbiological practice and the supervision of junior personnel;
- maintain a register of approved projects with their assessment as well as projects exempted under Sections 2.2.1(i) to 2.2.1 (vii) of these guidelines;
- maintain a list of the personnel who work in containment facilities, and ensure that new workers are familiar with the appropriate containment procedures and the correct use of laboratory equipment.

- take responsibility for drafting rules and making decisions about specific procedural safety matters. GMAC does not need to be consulted about these, as long as they are consistent with these guidelines.

5.2.7 Reporting Requirements

When the IBC is being set up it should provide GMAC with a completed 'Annual Report by Institutional Biosafety Committee' form. This form can be found in Appendix 3. The deadline for the annual submission is 1st January.

5.2.8 Medical Surveillance of Workers

For personnel using GM-BSL2 physical containment facilities, GMAC advises that no special arrangements are necessary outside the normal institutional practices for laboratory workers.

Institutions doing microbiological research may take baseline serum samples from personnel. Such samples are stored for diagnostic tests on workers exposed to accidents or who develop unexplained illness.

For experiments requiring a physical containment level of GM-BSL3 or higher, laboratory workers should have an initial medical examination, and other requirements as stipulated under the WHO Laboratory Biosafety Manual.

5.2.9 Accidents and Incidents

The IBC or the Biological Safety Officer should record both the accident or incident and the follow-up action. If the IBC Chair is satisfied that the accident or incident was directly attributable to genetic manipulation work, and was significant, they should make a report to both the GMAC and the head of the institution. An example of such an incident could be the intentional failure to comply with these guidelines, or an incident which might have risked human health or the environment. Under the WSHA, it may be required for such incidents to be reported to the MOM and/or relevant regulatory agencies.

5.3 PRINCIPAL INVESTIGATORS

The Principal Investigator should be thoroughly familiar with the requirements of these guidelines and should ensure that, the guidelines are adhered to, for any project he/she is responsible and for which, involves the use of genetic manipulation. Specifically, he/she should:

- assess the proposal to decide if it falls within the guidelines. If unsure, the investigator should consult the IBC;
- provide all information on the proposal that the IBC may need for assessment or monitoring of the proposal;
- follow through on GMAC's and IBC's advice and recommendations;
- fill out a typed original GMAC 'Proposal Form for Assessment of Genetic Manipulation Work' and hand a copy (keeping a second copy) to their IBC,

before starting work on any project which falls under these Guidelines, and ensure that work does not commence until approval is granted by the IBC;

- submit a new proposal form to the IBC before any major change is made to the experimental system of a proposal, which may result in a change of category or which may affect the exempt status of Category C projects.
- conduct experiments under the conditions of physical containment approved by the IBC or as advised by the GMAC in the case of category A proposals (see Section 4);
- ensure that students, subordinates, and other co-workers are aware of the nature of potential hazards of the work and have been given relevant training. They should also arrange for training, if needed;
- inform the IBC of any changes to the project team;
- promptly report accidents, unexplained illnesses and absences to the IBC;
- advise the IBC when intending to import biological material(s) falling within these guidelines (see also Section 6.7).

SECTION 6: IMPORT, EXPORT AND TRANSPORT FOR GMOs AND/OR GMO-DERIVED MATERIALS

6.1 GENERAL CONSIDERATIONS FOR TRANSPORT AND PACKAGING OF MATERIAL

6.1.1 Basic Requirements

The basic requirement for transport of viable genetically modified organisms is that there should be a minimal risk to humans, animal, plants and the environment.

Risk is minimized through a) proper packaging that will withstand rough handling and contain liquid material within the package without leakage to the outside; b) labelling of the package with the biohazard symbol and other labels to alert the workers in the transportation chain to the hazardous contents of the package; c) documentation of the hazardous contents of the package should such information be necessary in an emergency situation; and d) training of workers in the transportation chain to be able to respond appropriately to emergency situations.

The samples should be transported in packagings that correspond to their respective GMAC risk categories. The recipients should have facilities to contain the organisms at the required level.

6.1.2 Transport Requirements

For transport within an institution, standard operating procedures (SOP) implemented by the facility operator should include risk assessment and packaging according to risk category. Generally, GMOs of categories A and B that are transferred out of a containment laboratory must be triple packaged in sealed unbreakable containers or bags.

For transport outside an institution, procedures must have been set up for the safe transport of biological materials by air, rail and road. Packaging and transport arrangement should correspond to its risk level. Risk levels are categorised according to GMAC risk classification- Category A, B and C (See Section 4).

It is the responsibility of the sender and the recipient to make sure that all packaging and transport regulations are followed.

The transport of biological materials is controlled by the following regulations. The latest version should be consulted:

- The IATA *Dangerous Good Regulations* are comprehensive and incorporate the many provisions of the other regulations.

The IATA *Live Animals Transport Regulations* is the worldwide standard for transporting live animals and arthropods; they aim to ensure that both safety and animal welfare are addressed during transportation.

The International Air Transportation Association (IATA), *Dangerous Goods Regulations* and *Live Animals Transport Regulations*; (IATA online store – <http://www.iata.org/>)

- The Biological Agents and Toxins (Transportation) Regulation

In Singapore, the BATA regulates transportation of specified biological agents and toxins covered under the legislation (MOH Regulatory Policy Branch- <http://www.biosafety.moh.gov.sg/>).

6.1.3 Risk Classification

For the purpose of transport, biological materials are classified into different risk categories as follows:

Table 1: Risk Classification for transport of GM biological agents

Risk Classification	Characteristics of Biological Materials	Packaging Specifications	Regulatory Bodies
Category A	GMOs and GMMs that are 1) infectious and pathogenic to humans and/or animals and plants, 2) pose high risks to operators, the community or the environment and are 3) biological agents or toxins that are defined as First, Second and Fifth Schedule of the BATA and/or classified as Risk Group 3 and 4 of the WHO Laboratory Biosafety Manual.	Infectious materials should be transported in packaging that complies with the IATA Dangerous Goods Regulations. For live GM animals/arthropods, they may only be transported in packaging that complies with Container Requirement 62 of the IATA Live Animals Regulations. Transportation of First, Second and Fifth Schedule of the BATA should comply with the packaging and transportation requirements stated in the Biological Agents and Toxins Transportation Regulations.	Agri-Food & Veterinary Authority (AVA), Import and Export Regulation Division; Ministry of Health (MOH), Regulatory Policy Branch; National Environmental Agency (NEA), Environmental Health Department.
Category B	GMOs and GMMs that 1) are non-infectious and non-pathogenic to humans and/or animals and plants, 2) pose	GMMs and GMOs should be transported in packaging that complies with the IATA Dangerous Goods Regulations.	Agri-Food & Veterinary Authority (AVA), Import and Export

	low-level risks to operators, the community or the environment and 3) requires BSL2 containment or above for safe handling.	For live GM animals/arthropods, they may only be transported in packaging that complies with Container Requirement 62 of the IATA Live Animals Regulations. Transportation of BATA Third Schedule biological agent in quantities aggregating 10 litres or more should comply with the packaging and transportation requirements stated in the Biological Agents and Toxins Transportation Regulations.	Regulation Division; Ministry of Health (MOH), Biosafety Legislation Branch; National Environmental Agency (NEA), Environmental Health Department.
Category C	GMOs and GMMs that are 1) non-infectious and non-pathogenic to humans and/or animals and plants 2) do not pose significant risk to operators, the community or environment and 3) can be handled at BSL1 containment.	May be subjected to the provision of the transport regulations.	Agri-Food & Veterinary Authority (AVA), Import and Export Regulation Division

Packaging and labelling requirements should comply with the Transportation Regulations of the BATA, which are available under the public information of the MOH Biosafety Website.

Packaging of imports transported via air shall adhere to the packaging instructions of the International Air Transport Association (IATA). The above regulations detail: the certification requirements; the maximum quantities that can be transported by cargo or passenger aircraft; the external labelling requirements (including the identifying UN number); and the details to be included in the Shipper's Declaration for Dangerous Goods. Please refer to the International Air Transportation Association (IATA) for packaging specifications.

6.1.4 Documentation

When infectious material is being transported, a Shipper's Declaration for Dangerous Goods must be completed indicating information including origin, contents and date of dispatch, and should be placed in a separate leak-proof bag so as to protect the declaration form from potential contamination by the contents of the package. Recipients should be informed, before delivery, of all known hazards associated with the material.

6.1.5 Labelling

The package must be labelled (according to IATA standards) to clearly show the name, address and contact details of the persons responsible for the materials, so that the person can be contacted should the package be lost, damaged or misdirected.

6.1.6 General

- Only trained personnel may undertake the packaging for transport which should be done according to the above regulations.
- Facilities should be provided for after-hours delivery of samples, and all staff including night staff should be warned of any hazards.
- Procedures and precautions for unpacking should be appropriate to the type of package being unpacked.
- When infectious waste is removed from a laboratory, waste should be disposed of by waste disposal contractors licensed by NEA and in accordance to NEA guidelines on disposal of such waste.

6.2 TRANSPORT OF GENETICALLY MODIFIED MICROORGANISMS

6.2.1 General considerations

- All genetically modified microorganisms should undergo risk assessment (See Table 1) to determine its risk category.
- The transport and packaging of any First, Second, Third and Fifth Schedule biological agents regulated in the Biological Agents and Toxins Act (BATA) in Singapore should comply with the Transportation Regulation of the BATA. No person shall transport or procure the transportation of any biological agent or toxin under the BATA within Singapore by mail or public transport.
- Transport of biological agents via air shall adhere to the specified packaging under the IATA Dangerous Good Regulations.
- Both sender and addressee need to ensure that no sample vials/canisters containing GMMs are missing in the delivery process. It is the responsibility for both sender and addressee to exercise diligence **respectively** in checking the number of sample vials/canisters containing the GMMs tally at the point of delivery to that received

6.2.2 Packaging and Transport Requirements

- Transgenic or genetically modified microorganisms (GMMs) to be transported must be wholly contained inside a watertight, leak-proof, sealed, unbreakable primary container packed in a secondary container and finally packed into a rigid outer container.
- Sufficient quality of absorbent material should be wrapped around the primary container to absorb all fluids (if any) that may emanate from the biological agent in the event of a breakage of or leakage from the primary containers.
- The packaging should be sufficiently strong to withstand any impact which the package would normally be subject to during the transportation, loading and unloading.

6.2.3 Labelling

- The outermost container must be labelled to clearly show the species/origin of the GMM, the name, address and contact details of the person responsible for the dealings, so that the person can be contacted should the package be lost, damaged or misdirected.
- A biohazard label must be attached to at least the outermost container holding any GMOs which fit into the classification of Cat A and B.

6.2.4 Treatment of containers after transport

Please refer to Appendix 19 for disposal and decontamination requirements.

Any materials transported with the GMMs must be either retained with the organisms under containment or decontaminated after transport has occurred.

6.3 TRANSPORT OF TRANSGENIC ANIMALS

6.3.1 General considerations

In making transport arrangement for transgenic animals, two principles are paramount:

- the need to prevent the animals from escaping, to ensure that transgenic animals will not interbreed with feral populations; all reasonable scenarios such as accidents should be considered;
- the need to ensure that the animals are properly identified, that they arrive at the intended destination, and that a competent biologist with experience in handling transgenic animals takes delivery of them. Accounting procedures should be instigated to make sure that all animals sent are delivered - whether dead or alive.

6.3.2 Packaging and Transport requirements

- The IBC should formulate rules it considers essential to meet these conditions. It may be necessary for the IBC to inspect the transport arrangements to determine that the above principles are complied with and that any additional conditions which the IBC considers appropriate have been met (see also Appendix 12).
- Animal boxes should comply with IATA standards. Modifications can be made to the boxes especially for pathogen-free animals. The boxes must be escape-proof and allow easy observation during an import inspection without opening the box.
- Transport of all animals should also adhere to the NAELAR Guidelines on the Care and Use of Animals for Scientific Purposes.

6.4 TRANSPORT OF TRANSGENIC ARTHROPOD AND THEIR PATHOGENS

Genetically manipulated arthropods (including live arthropods and arthropod cell cultures infected with genetically manipulated pathogens and microorganisms)

6.4.1 General Considerations

- Transport of live transgenic arthropods requires packaging that prevents the escape of the arthropods, maintains their viability and protects personnel in the transportation chain from exposure to the contents.
- The selection of transport packaging begins by establishing the phenotypic change in the arthropod and/or microorganism due to genetic modification, and potential impact of escaped transgenic arthropods.
- Transport packaging is directly correlated with its risk of infection, pathogenicity and transfer of transgenes when handled by operators in the transport chain and in the event of accidental release.
- Accounting procedures should be instigated to make sure that the same number of organisms and containers that are sent are delivered.

6.4.2 Packaging and Transport Requirements

- Transport packaging should prevent leakage/escape of the contents; unbreakable, able to tolerate pressure changes and other conditions incident to ordinary handling in transportation.
- It is recommended that this packaging consist of three layers of containment; a sealed primary receptacle surrounded by padding enclosed in a solid, transparent, unbreakable secondary container with the lid tightly secured and lastly in an appropriate transport container.
- If aquatic stages are transported, the container should also contain sufficient absorptive material to absorb and contain all of the water.

- IATA Live Animals Regulation 26th Edition (LARs) describes containers that are appropriate for the shipment of arthropods (See Container requirements 62 of LARs).
- Upon arrival, the arthropods should immediately be transferred from the holding container to a new container.

6.4.3 Labelling

- The outermost container should bear a 'biohazard label' and must be labelled to clearly show the species, strain/origin of the transgenic arthropods.
- The label must include the name, address and contact details of the person responsible for the transgenic arthropods, so that that person can be contacted should the package be lost, damaged or misdirected.

6.4.4 Treatment of containment after transportation

Please refer to Appendix 19 for disposal and decontamination requirements.

- After transfer of the transported arthropods into new containers, all of the transport material should be decontaminated by autoclaving or incinerated.

6.5 TRANSPORT OF TRANSGENIC PLANTS

6.5.1 Packaging and Transport requirements

- Vegetative transgenic plant material should be transported within and between institutions in a primary container (for example, a plastic bag for cuttings, an envelope for seeds), which is packed in another unbreakable container.
- Whole transgenic plants should be netted and deflowered and all seed or fruit removed before transport. Plants may be transported in pots, contained in boxes or crates.

6.5.2 Labelling

- The outer container should be labelled to indicate that it contains transgenic plant material, and the label should include the telephone number of a person to contact should the package be lost or damaged. Labels on seed packets should include the number of seeds being transported.

6.5.3 Documentation

- Accounting procedures should be instigated to make sure that the same number of plants and containers that are sent are delivered.

6.6 SUPPLY OF GENETICALLY MANIPULATED MATERIAL TO OTHER RESEARCH WORKERS

- 6.6.1 Investigators supplying people in Singapore with genetically manipulated material shall make sure that recipients who are unfamiliar with the genetic manipulation field are made aware of the existence of these guidelines and of the need to observe them. If the genetically manipulated materials fall under the First, Second and Fifth Schedule of the BATA, MOH shall be notified of the transfer of the materials.
- 6.6.2 When genetically manipulated animals are supplied to investigators overseas, the requirements of the export legislations of Singapore must be met. After obtaining an AVA export license, the exporter should apply for an export permit (strategic goods export permit if the export is subject to Strategic Goods (Control) Act) through TradeNet® (Link can be found on URL: <http://www.customs.gov.sg>) prior to exportation.

For strategic goods export procedures:

Singapore Customs
55 Newton Road, #07-02 Revenue House, Singapore 307987
Tel: 63552000
Fax: 62509606
Email: customs_stgc@customs.gov.sg
Website: <http://www.customs.gov.sg/stgc>

For export of laboratory animals:

Import & Export Regulation Division
Quarantine & Inspection Department
Agri-Food & Veterinary Authority
5 Maxwell Road, #02-03, Tower Block, MND Complex, Singapore 069110
Tel: 6227 0670 or 6325 7333
Fax: 62270670
Email: AVA_Import&Export_Animals@ava.gov.sg
Website: www.ava.gov.sg

To make sure that Singapore's responsibility as an exporter, individuals or organisations exporting genetically manipulated organisms must include the following information to authorities in the recipient country.

- details of the risk assessments that have been carried out in Singapore, and the conditions under which the organism has been approved for use in Singapore (e.g. contained use in a BSL2 laboratory, or small scale field trial under specified conditions);
- any information known to the sender about possible adverse effects of the organism in the recipient country. (In many cases, a disclaimer that no assessment has been made of potential effects in the recipient country may be suitable.)

6.7 IMPORT OF GENETICALLY MANIPULATED ORGANISMS OR MATERIAL

- 6.7.1 Importation or possession of GMOs, not relating to human health, is regulated under Section 9 of the Animals and Birds Act (Cap 7) and Part IV of the Control of Vectors and Pesticides Act (Cap 59).
- 6.7.2 Importation or procurement to import specific biological agents and toxins, which is capable of causing death, disease or malfunction in a human, as those specified under the First, Second, Fourth and Fifth Schedules, is regulated under the Biological Agents and Toxins Act (Act 36 of 2005).
- 6.7.3 Work with imported genetically manipulated viral genomes, micro-organisms, arthropod, plants or animals fall under the extent of these Guidelines. Investigators should seek the approval of the relevant regulatory agency for intended import and submit a proposal for assessment if appropriate. Regulated organisms are stated in the Bio-security Assurance Arrangement Guidelines and the Notifiable Diseases list of the Animal and Birds Act of the Agri-food and Veterinary Authority (AVA). The import of any arthropod vectors capable of transmitting infectious diseases regulated in the Infectious Diseases Act (Cap 137) of the National Environmental Agency (NEA).
- 6.7.4 Permission should be obtained from the relevant agency to import all biological materials, including transgenic micro-organisms, plants, animals and arthropods. After obtaining the permission from the relevant agencies, the importer should apply for an import permit through TradeNet® (Link can be found on URL: <http://www.customs.gov.sg/>). Permission can be obtained by submitting an 'Application for Import Permit' to the appropriate quarantine officer given on the form. The form is available from:

For Plants, Animals, plant-related or animal-related pathogens and pests:

Import & Export Regulation Division
Quarantine & Inspection Department
Agri-Food & Veterinary Authority
5 Maxwell Road, #02-03, Tower Block, MND Complex, Singapore
069110

Tel: 6227 0670 or 6325 7333
Fax: 62270670
Email: AVA_Import&Export_Animals@ava.gov.sg
Website: www.ava.gov.sg

For Human related pathogens:

Regulatory Policy Branch, Ministry of Health (MOH)
College of Medicine Building, 16 College Road, Singapore 169685

Tel: 6325 8459/ 9205/ 8605
Fax: 6325 9072
Email: MOH_Biosafety@moh.gov.sg
Website: <http://www.biosafety.moh.gov.sg>

For vectors² (arthropod or rodent) carrying or causing any disease to human beings:

Head, Operations
Environmental Health Department, National Environment Agency (NEA)
40 Scotts Road, Environment Building #21-00
Singapore 228231

Tel: 67319314

6.7.5 The information provided should contain any relevant information about the genetically manipulated material which falls within these Guidelines. In particular, the following information should be included if appropriate:

- details of the donor organism, which is the DNA source and characteristics of the genes transferred;
- the method or vector used to transfer the DNA to the host organism;
- host organism;
- complete details should be provided if a plant or animal is infected with a genetically manipulated micro-organism.
- Risk assessment on import, handling and transport of the genetically manipulated material.

6.7.6 If appropriate, the relevant agency may make the information provided on this form available to the GMAC, or to other assessors, and may request specific information about any aspect of the genetically manipulated material. GMAC's assessment of the Application to Import will be accelerated if a small-scale proposal for the work has already been received.

² "Vector" means any arthropod, including its egg, larva and pupa, and any rodent, including its young, carrying or causing, or capable of carrying or causing any disease to human beings.

APPENDICES

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APPENDIX 1: OTHER RELEVANT DOCUMENTS

Documents relevant to and mentioned in the guidelines are listed below.

1. Relevant Local Legislation

The requirements of the regulatory bodies should be met. All local legislation is available on the Singapore Statutes Online [<http://statutes.agc.gov.sg/>]

- 1.1. Animal and Birds Act (Cap 7)
- 1.2. Biological Agents and Toxins Act 2005 (Act 36 of 2005)
- 1.3. Control of Plants Act (Cap 57A)
- 1.4. Workplace Safety and Health Act 2006 (Act 7 of 2006)

2. Relevant Local Guidelines

2.1. Singapore Guidelines on the Release of Agriculture-Related GMOs, GMAC

The publication is available from:

- GMAC Secretariat or
- As a downloadable PDF from the GMAC website at <http://www.gmac.gov.sg>

2.2. Laboratory Biosafety Manual, 3rd edition, World Health Organization, 2004, (ISBN 92-4-154650-6); relevant to the Biological Agents and Toxins Act

Publications of the WHO can be obtained from:

*Marketing and Dissemination, World Health Organization
20 Avenue Appia, 1211 Geneva 27, Switzerland
Email: bookorders@who.int*

2.3. Guidelines on the Care and Use of Animals for Scientific Purposes, NACLAR

The publication is available from:

- The National Advisory Committee for Laboratory Animal Research
NACLAR Secretariat
Centre for Maritime Studies
12 Prince George's Park, S118411 OR
- As a downloadable PDF from <http://www.ava.gov.sg/AnimalsPetSector/CareAndUseAnimalsForScientificPurp/>

3. Worldwide guidelines specific to genetic manipulation

3.1. Australia

- i. Guidelines for Certification of Facilities/Physical Containment Requirements
- ii. Good Industrial Large Scale Practice
- iii. Guidelines for the transport of GMOs

Publications (i) – (iii) above are available from:

- Office of Gene Technology Regulator
Commonwealth Department of Health and Aging
MDP54 PO Box 100 Woden
ACT 2606, Australia
- <http://www.ogtr.gov.au/>

3.2. United States of America

- i. Guidelines for Research Involving Recombinant DNA Molecules, National Institutes of Health, Department of Health and Human Services, USA

The publication is available from:

- Office of Biotechnology Activities
National Institutes of Health, MSC 7010
6000 Executive Blvd, Suite 302
Bethesda, MD 20892-7010
United States of America
- <http://oba.od.nih.gov/oba/index.html>

3.3. European Commission

- i. Guidelines for the Risk Assessment of Operations Involving the Contained Use of Genetically Modified Micro-organisms (GMOs), September 1993
ACGM/HSE/DOE/NOTE 7
- ii. Laboratory Containment Facilities for Genetic Manipulation, June 1988
ACGM/HSE/NOTE 8

Publications (i) and (ii) above, and other 'Notes' that comprise the UK Guidelines can be obtained from:

- Advisory Committee on Genetic Modification
Health and Safety Executive
Rose Court, 2 Southwark Bridge
London SE1 9HS, United Kingdom
- <http://www.hse.gov.uk/index.htm>

- iii. Safety Considerations for Biotechnology 1992, OECD, Paris, 1992

Publication (iii) above is available from:

- OECD Publications Service
2 rue André-Pascal
75775 Paris, Cedex 16
France

4. Other relevant guidelines, regulations and publications

- i. Australian Code of Good Manufacturing Practice for Therapeutic Goods – Medicinal Products
- ii. Australian Guidelines for the Registration of Drugs, Vol. 1, 2nd edition, July 1994 (Includes Appendix 17: Guidelines on clinical data to support applications for substances produced by genetic manipulation; and Appendix 18: Guidelines on clinical data to support applications for monoclonal antibodies.)

Publications (i) and (ii) above are available from:

- *Publications Officer, Therapeutic Goods Administration
PO Box 100, Woden ACT 2606
Australia*
- <http://www.health.gov.au/>

- iii. Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, NHMRC, CSIRO, Australian Agricultural Council, 1997, ISBN 0-644-10292-6
- iv. NHMRC Statement on Human Experimentation and Supplementary Notes, 1992
- v. Human Gene Therapy and Related Procedures, 1994, ISBN 0-644-32916-5
- vi. National Guidelines for the Management of Clinical and Related Wastes, 1988, ISBN 0-644-08265-8

Publications (iii) – (vi) above are available from:

- *The Publications Officer
National Health and Medical Research Council
GPO Box 9848, Canberra ACT 2601
Australia*
- <http://www.nhmrc.gov.au/>

- vii. Laboratory Safety Guidelines that Take Account of HIV and Other Blood-Borne Diseases, ANCA Bulletin No 3, January 1990
- viii. Infection Control in the Health Care Setting - Guidelines for the Prevention of the Transmission of Infectious Diseases, April 1996

Publications (vii) – (viii) above are available from:

- *Australian National Council on AIDS and Related Diseases
GPO Box 9848, Canberra ACT 2601
Australia*

- ix. AS 2243: Safety in laboratories
- x. AS/NZS 2243.1: 1997: Safety in laboratories: General
- xi. AS/NZS 2243.3: 1995: Safety in laboratories: Microbiology and Amdt 1, April 1996
- xii. AS 2252: Biological safety cabinets
- xiii. AS 2252.1: 1994: Biological safety cabinets (Class I) for personnel protection and environmental protection

- xiv. AS 2252.2: 1994: Laminar flow biological safety cabinets (Class II) for personnel, environment and product protection
- xv. AS 2647: Biological safety cabinets - installation and use, 1994
- xvi. AS 2982: Laboratory design and construction
- xvii. AS/NZS 2982.1: 1997: General requirements
- xviii. AS 1324: Air Filters for use in air conditioning and general ventilation
- xix. AS 1324.1: 1996: Construction
- xx. AS 1324.2: 1996: Tests
- xxi. AS 1807.6: Cleanroom, workstations and safety cabinets – Methods of test: Determination of integrity of terminally mounted HEPA filter installations , 1989

Publications (ix) – (xxi) above are available from:

- Standards Association of Australia
National Sales Centre PO Box 1055
Strathfield NSW 2135
Australia

- xxii. The requirements manual for agricultural chemicals
- xxiii. The requirements manual for veterinary chemicals

Publications (xxii) – (xxiii) above are available from:

- AGPS Mail Order Sales
GPO Box 84, Canberra ACT 2601
Australia

- xxiv. Vaccination of Laboratory Workers Handling Vaccinia and Related Poxviruses Infectious for Humans, 1990, Advisory Committee on Dangerous Pathogens and Advisory Committee on Genetic Modification, ISBN 0-11-885450-X
- xxv. HIV - the Causative Agent of AIDS and Related Conditions, January 1990
Advisory Committee on Dangerous Pathogens

Publications (xxiv) – (xxv) are available from:

- Health and Safety Executive
Rose Court, 2 Southwark Bridge
London SE1 9HF, United Kingdom
- HMSO Publications Centre
PO Box 276, London SW8 5DT
United Kingdom

- xxvi. Biosafety in Microbiological and Biomedical Laboratories , 4th edition, Washington DC, 1999, US Department of Health and Human Services

Publication (xxvi) – (xxv) above is available from:

- Superintendent of Documents
US Government Printing Office
Washington DC 20402, United States of America
- <http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm>

xxvii. Collins C H, Laboratory Acquired Infections: History, Incidence, Causes and Prevention, 3rd edition, Butterworths-Heinemann, London , 1993, ISBN 0-750606428

xxviii. *Convention on Biological Diversity* , June 1992, United Nations Environment Programme

The above publication is available from:

- Secretariat, Convention on Biological Diversity
United Nations Environment Programme
15 chemin des Anemones
CP 356, CH-1219 Chatelaine, Geneva
Switzerland
- <http://www.cbd.int/>

xxx. Information Systems for Biotechnology

The above publication is available from:

- <http://www.nbiap.vt.edu/>

xxxi. Arthropod Containment Guidelines (Version 3.1)

The above publication is available from:

- The American Society of Tropical Medicine and Hygiene -
<http://www.astmh.org>

APPENDIX 2: LIST OF GMAC-APPROVED HOST/VECTOR SYSTEMS**BIOLOGICAL CONTAINMENT**

The objective of biological containment is to minimise both the survival of the host and vector outside the laboratory, and the transmission of the vector from the propagation host to a non-laboratory host. This Appendix lists the host/vector systems currently approved by GMAC as providing a level of biological containment.

	Host	Vector
Bacteria	<i>Escherichia coli</i> K12 or <i>E. coli</i> B derivatives which do not contain conjugative or generalised transducing phages	1. Non-conjugative plasmids 2. Bacteriophage - lambda - lambdoid - Fd or F1 (e.g.M13)
	<i>Bacillus subtilis</i> or <i>B. licheniformis</i> Asporogenic strains with a reversion frequency of less than 10^{-7}	Indigenous <i>Bacillus</i> plasmids and phages whose host range does not include <i>B. cereus</i> or <i>B. anthracis</i>
	<i>Pseudomonas putida</i> Strain KT 2440	Certified plasmids: pKT 262, pKT 263, pKT 264
	<i>Streptomyces</i> -specified species: <i>S. coelicolor</i> <i>S. lividans</i> <i>S. parvulus</i> <i>S. griseus</i>	1. Certified plasmids: SCP2, SLP1, SLP2 PIJ101 and derivatives 2. Actinophage phi C31 and derivatives
Fungi	<i>Neurospora crassa</i> , laboratory strains	No restriction
	<i>Saccharomyces cerevisiae</i>	No restriction
	<i>Pichia pastoris</i>	No restriction
	<i>Schizosaccharomyces pombe</i>	No restriction
Slime moulds	<i>Dictyostelium</i> species	<i>Dictyostelium</i> shuttle vectors, including those based on the endogenous plasmids Ddp1 and Ddp2

	Host	Vector
Tissue Culture	Mammalian (including human) cells	1. Non-viral vectors or defective viral vectors (including retrovirus or retroviral-helper combinations) that cannot infect human cells. 2. Advanced generation of lentiviral vector ³(3rd generation and later)
	Avian cells	Avipoxvirus vectors
	Plant cell cultures	Non-tumorigenic disarmed Ti plasmid vectors in <i>Agrobacterium tumefaciens</i> and non-pathogenic viral vectors
	Insect cell cultures, such as <i>Spodoptera frugiperda</i> ^{4*}	Baculovirus (<i>Autographa californica</i> nuclear polyhedrosis virus)

Note:

The above approved hosts may also be used in experiments where DNA is inserted into the host cell without the use of a biological vector (for example, by mechanical, electrical or other means), provided that the DNA:

- is not derived from microorganisms able to cause disease in humans, animals or plants, unless the DNA to be introduced is fully characterised and will not increase the virulence of the host or vector;
- does not code for a toxin for vertebrates with an LD50 of less than 100 µg/kg, and is not an oncogene;
- does not comprise or represent more than two-thirds of the genome of a virus and is not being used in an experiment in which the genetic material missing from the viral genome and essential for producing infection is available in the cell into which the incomplete genome is introduced, or made available by subsequent breeding processes.
- Any commercially available Host-Vector Systems.

Such a system with an approved host and the DNA meeting these conditions would constitute an approved host/vector system for the purposes of these Guidelines, and hence would fall under exemption section 2.2.1.

³ Please refer to Appendix 20 for more details. For 'Advanced lentiviral vector' definition, please refer to the MOH Regulatory Policy Branch – <http://www.biosafety.moh.gov.sg>

⁴ provided the recombinants are also inclusion-negative (e.g. polyhedrin minus)

APPENDIX 3: INSTRUCTIONS FOR COMPLETING GMAC PROPOSAL FORMS AND ANNUAL REPORT BY IBC

The Institutional Biosafety Committee will use the information provided in the GMAC 'Proposal Form for Assessment of Genetic Manipulation Work' to determine the category into which the project fits and the containment level. GMAC will use the information in the form to assess proposals falling into Category A. In order to enable GMAC and the IBC to carry out those functions, a clear statement of what the applicant proposes to do is needed, and if this cannot easily be fitted into the space provided, a separate description shall be attached.

If there are no changes made to the purpose of the project, protocol, vectors and transgenes used in a previously endorsed proposal that has expired, the GMAC "Proposal Form for Extension of GMAC Endorsement" can be used to extend the endorsement administratively; without risk assessment by GMAC.

The following suggestions are intended to ensure that GMAC has sufficient relevant information to make a prompt decision on the application.

Title of Project and Aims

When describing the aim of the proposal, include a brief description of the main steps involved. If both immediate and long term broader aims are included, make clear which component of the work needs IBC approval (or GMAC advice) now.

If the project is complex and likely to take several years to complete, it will help if the work to be undertaken first is described separately. If recombinant DNA is ultimately to be inserted into whole animals or plants, or into bacteria not listed in Appendix 2 of these Guidelines, it may well be that approval can be given in the first instance for cloning and characterisation of specific genes or other DNA, with approval for later stages being possible only after such characterisation. If the stages are made clear to the Committee, approval or advice for the first stage may be given to enable the work to start.

If the intention is to import biological material which falls under the Guidelines, the title may read 'Intention to import...'.

Source of DNA

If the DNA has already been cloned, please give details of the construct: e.g. who made it, how it was made, and what is known of its properties.

If several genes or species etc. are to be used, list all of them, because one proposal may cover them all. For example, if appropriate, request advice for chickens, ducks and other avian species. This will alleviate the need for repeated applications.

Host Organism

If more than one host is to be used, particularly if different containment levels apply, clarify when and how each is to be used. Comments made above on dividing a project into stages may be relevant. Please also complete the supplementary information form for experiments involving whole plants.

Vectors

Make the description for prokaryotic vectors as broad as is necessary to cover the intended work. For example, specify 'non-conjugative plasmids such as pBR322 and pUC9' if the project is likely to require a range of specific vectors as the project progresses. If only pBR322 and pUC9 are requested, the approval will be limited to the two vectors and will not cover the many closely related vectors which may turn out to be more useful.

The description of vectors should comprise more than a series of letters, symbols and numbers. Some description of their properties is also required.

In the case of retroviral vectors, be specific and indicate what is known about their properties, and provide details of the construct, if appropriate, on a separate sheet of paper, including a genetic map and/or a description of its construction.

Assessment of Potential Hazards

The increasing range of hosts and vectors being used makes it imperative for every applicant to assess assiduously any potential hazards associated with the proposed work and to discuss such hazards and their containment in the proposal. Failure to do so may delay assessment until after additional information has been sought and obtained from the applicant.

Details of Personnel

For the 'full details' requested, please include the extent and nature of relevant experience on a separate sheet of paper. These details need not be sent to GMAC but should be checked by the IBC.

Proposal Form

A copy of the proposal form is attached to this Appendix. The proposal form is self-duplicating and allows for sufficient copies to be made for GMAC, the IBC and the principal investigator's records. Please note that GMAC requires the originals (i.e. top copies) of forms to be submitted, and not photocopies.

PROPOSAL FORM FOR ASSESSMENT OF GENETIC MANIPULATION WORK

GMAC Ref No.: _____
(For official use only)

Name of Scientist(s) : _____

Name of Institution : _____

Type of Experimental Organisms (please check the appropriate box) :

☐ Animal

☐ Plant

☐ Others, please specify: _____

Experiment Risk Group (please check the appropriate box) :

☐ Category A

☐ Category B

☐ Category C

A. Experimental detail (attach separate sheet if necessary)

1.	Project title
2.	Research unit involved
3.	Experimental objective
4.	Rationale for the experiment

5a.	Description of transgene(s) and gene construct(s)
5b.	Nature of Transgene(s) [please check the appropriate box]: ☐ Oncogenic ☐ Toxic ☐ Non-oncogenic and non-toxic
6.	Method of gene delivery
7.	Duration of the experiment (≤ 3 years)

B. Organism/vector (attach separate sheet if necessary)

1.	Name and address of exporting user
2.	Date of transfer
3.	Name of organism/vector
4.	Natural host
5.	Requirement(s) to ensure containment, safe handling, storage and disposal

Prepared by:

Name and Signature	Appointment / Laboratory	Date
Contact Details		
Address :		
Tel Number :		Fax Number :
Email :		

Reviewed by:

Date received	Name and Signature	Date
IBC Chairman		

Note that IBCs should provide annual reports of its composition and activities by 1st January every year.

The following section is applicable for Category A experiments only:

Please indicate if approval have been sought from relevant regulatory authority (MOH/AVA/NEA) for *use/possession/import/transport of the GMO.

If yes, please provide supplementary proof.

If no, please explain why.

**(highlight where applicable)*

For a list of regulatory contact points, please refer to Section 6.6 (page 23).

PROPOSAL FORM FOR EXTENSION OF GMAC ENDORSEMENT

GMAC Ref No.: _____

Your Ref No. : _____
(For official use only)

Name of Scientist(s) : _____

Name of Institution : _____

Extension of GMAC Endorsement

For extension of GMAC endorsement, please provide the following details:

- i. GMAC Reference Number of previous proposal : _____
- ii. Extension Period (please check the appropriate box):
☐ 1 year ☐ 2 years ☐ 3 years

I, _____ state that there is no changes in the purpose, genes of interest, vectors and protocols that was previously submitted and endorsed by GMAC.

Prepared by:

Name and Signature

Appointment / Laboratory

Date

Contact Details

Address : _____

Tel Number : _____ Fax Number : _____

Email : _____

Reviewed by:

Date received

Name and Signature

Date

*Please note that IBCs should provide annual reports of its composition and activities by 1st January every year.

SUPPLEMENTARY INFORMATION FORM
FOR EXPERIMENTS INVOLVING WHOLE PLANTS
(Attach separate sheet if necessary)

1. Are the experimental plant noxious weeds or closely related to species which are noxious weeds?

If 'yes', please elaborate:

2. Are the microorganisms/fungi etc. involved in this work known to be harmful to humans, animals or plants?

If 'yes' :

a) Give further information about the harmful agent:

b) Detail the known and likely transmission modes (including carrier insects) for this agent:

3. Are the genetically manipulated plants to be grown?

If 'yes' :

a) What developmental stage will they reach?

- b) Describe the techniques to be employed to contain plant materials (including pollen, seeds, spores, vegetative materials) during and at the completion of the experiments.
 - c) What is the proposed method of disposal of plant materials at the conclusion of the experiment?
- 4.
 - a) Is soil or soil substitute to be used? (Specify.)
 - b) How will it be sterilised?
- 5. Describe the facility to be used for cultivation of the plants. Include information such as location, proximity to containment laboratory etc.:
- 6. Give any additional information which may be relevant to the assessment of this work:

ANNUAL REPORT BY INSTITUTIONAL BIOSAFETY COMMITTEE

(Please submit to GMAC Secretariat by 1st January)

Name of Institution : _____

Address of Institution : _____

Tel : _____ Fax : _____

Submitted by : _____ Date: _____

Name and Signature

A. Composition of IBC

IBC APPOINTMENT	NAME	DESIGNATION
Chairman		
Biological Safety Officer		
Committee Members		

B. List of Current Proposals for Genetic Manipulation Work (attach separate sheet if necessary)

i) Category A

--

ii) Category B

iii) Category C

C. Report on Significant Accident or Incident attributed to Genetic Manipulation Work
(attach separate sheet if necessary)

D. List of Additional Comments for GMAC

List of Approved Facilities for Genetic Manipulation Work

(Please complete one copy for each facility with a containment level equivalent to BSL3, and for each large scale facility.)

Name of Facility : _____

Location : _____

Manager : _____

Special features of facility :

Date of Last Inspection: _____ Date of Previous Inspection: _____

Period of room use and projects for which it has been used :

Dates of Staff Training: _____ (Cleaning Staff)

_____ (Emergency Staff)

_____ (Engineering Staff)

List of Current Manuals and Inspection Date :

Inspection Dates for :

_____ (Air handling system)

_____ (Alarms)

_____ (Biosafety Cabinet)

_____ (Building Finishes)

_____ (Centrifuges)

_____ (Cleanliness)

_____ (Decontamination Equipment)

_____ (Effluent Disposal)

_____ (HEPA filters)

_____ (Refrigeration)

_____ (Sterilisers)

Please keep all necessary documentation as proof for the above, to be provided when requested by GMAC or during on-site inspections.

APPENDIX 4: TOXINS

For work involving toxins that fall under Fifth Schedule of the BATA, prior approval for its use must be sought from the Ministry of Health.

Fifth Schedule Agents (as of 6th October 2011)

1. Botulinum toxins (Types A, B, C, D, E, F and G)
2. *Clostridium perfringens* toxins
3. Staphylococcal Enterotoxin B
4. Shigatoxins
5. T-2 Toxin
6. HT-2 Toxin
7. Tetanus Toxin
8. Verotoxins

Section 4.1, Category A(i), of these Guidelines requires that, for work involving the cloning of genes for toxins (or uncharacterised DNA from organisms that synthesise toxins for vertebrates) with an LD₅₀ of less than 100 µg/kg, the IBC seek a recommendation from GMAC before giving approval for work to commence.

Below is a list of a number of toxins with an LD₅₀ of less than 100 µg/kg. The list is not exhaustive. If it is not known whether a substance should be regarded as toxic, GMAC advice shall be sought.

SOME TOXINS WITH AN LD₅₀ OF LESS THAN 100 µg/kg⁵

Abrin

Bacillus anthracis lethal factor

Bordetella pertussis toxin

Cholera - see *Vibrio cholerae*

Clostridium botulinum toxins

Clostridium perfringens epsilon toxin

Clostridium tetani toxin

Corynebacterium diphtheriae toxins

Escherichia coli heat labile (LT) enterotoxin and LT-like toxin

Oxygen-labile haemolysins such as streptolysin O

Yersinia pestis murine toxins

Pseudomonas aeruginosa exotoxin A

Ricin

Shigella dysenteriae toxin

Staphylococcus aureus determinants A, B and F, alpha and beta toxin, exfoliative toxin

Vibrio cholerae toxin and toxins neutralised by antiserum monospecific for cholera toxin (e.g. heat labile toxins of *E. coli*, *Klebsiella* and other related enterotoxins)

Yersinia enterocolitica heat stable toxin

⁵ Information derived from the NIH Federal Register, Vol 51, No 88, May 1986 (Appendix F) and information provided by the NIH Office of Recombinant DNA Activities.

APPENDIX 5: GUIDANCE FOR WORK INVOLVING GM VIRUSES FOR GENE TRANSFER INTO ANIMAL & HUMAN CELLS IN A LABORATORY SETTING

1. General Considerations

Genetic modification of viruses is a common practice in medical research laboratories to study the biology of the viruses. Viruses are also used as efficient vehicles for gene transfer into animal and human cells.

The hazards associated with the use of genetically-modified viruses depend on the following:

- (a) The virus' host range;
- (b) Its ability for repeated rounds of infection;
- (c) Its competence for replication inside the cell;
- (d) The possibility of generating replication-competent virus from replication-defective vectors by recombination (e.g. in the case of retroviruses);
- (e) The ability of the genetic material of the virus to be integrated into the chromosome of the infected cell;
- (f) The stability of the virus inside the cell and exposed in the environment;
- (g) The means of transmission of the virus (e.g. through aerosol or skin abrasions and other physical contact);
- (h) The nature of the introduced DNA sequences or its encoded protein.

2. Approval and Consultation

Principal Investigators should seek the clearance from IBC prior to the start of any experimentation with genetically-modified viruses.

Exhaustive references for the Biosafety Level and Guidelines with regards to the use of specific animal and human-infectious viruses shall be obtained by the Principal Investigator prior to the start of the project. The following organisations provide good references for specific animal and human viruses:

- (a) American Biological Safety Association (ABSA)
www.absa.org
- (b) Center for Disease Control (CDC)
www.cdc.gov
- (c) American Tissue Culture Collection (ATCC)
www.atcc.org

3. Procedures for handling rodent and other non-human viruses

Viruses that are capable of infecting **ONLY animal (non-human and non-primate)** species are not considered to be associated with any hazard to the manipulator or other laboratory personnel. Practices associated with good tissue culture technique will be adequate in containing and handling these viruses. While the risks associated with the use of these viruses are considered negligible, solutions and contaminated cells shall be decontaminated/autoclaved before disposal to prevent accidental infection of other animal cell lines.

4. Procedures for handling viruses that can potentially infect human cells ('human infectious' viruses)

The primary hazard associated with the use of live recombinant viruses that have the capacity or that could potentially infect human cells lies on the type of the viruses and the nature of the introduced genetic sequences. Primate-infectious viruses shall be considered "potentially human-infectious". For all human-infectious viruses handled in a class II biological safety cabinet, the primary hazard to the scientist is the possibility of infection by viruses through broken skin brought about by needles and other commonly used sharps such as pipettes. The potential danger to other laboratory personnel depends on the stability and infectivity of the virus in the extracellular environment and the nature of the introduced genetic material.

For all human-infectious or potentially human-infectious viruses, the major requirement is for good virological and tissue culture practice on the part of the scientist with regard to the following precautions:

- (a) A facility of containment of level BSL2 or higher (as determined by the IBC and with reference to the Biosafety level detailed by the ATCC and CDC) will be required. All manipulations shall be conducted in a class II biological safety cabinet or equivalent. Only one individual shall use the cabinet at any one time.
- (b) Laboratory gowns, gloves and face mask shall be worn, as appropriate PPE during manipulations with recombinant human-infectious viruses.
- (c) Dishes and plates of cells containing human-infectious viruses shall be handled in larger plates (or inverted lids) to provide traps for accidental spills.
- (d) All pipettes, glassware and plasticware shall be decontaminated with an efficacious chemical disinfectant or autoclaved before disposal, taking into considerations sharps disposal requirements.
- (e) For viruses which are able to persist in the environment (e.g. adenovirus, vaccinia virus, hepatitis virus, papillomavirus), decontamination and bagging of waste should be done within the biosafety cabinet prior to removal and autoclaving. Care should be taken to ensure that the amount of material held in the biosafety cabinet is minimised, in order to avoid interference with the air flow in the cabinet.
- (f) Mouth pipetting is strictly prohibited.
- (g) Open flames that could interrupt the air-flow in the biosafety cabinet should be avoided.
- (h) The use of sharp instruments (sharps) such as syringe needles, glass pipettes, razors, scissors and surgical knives, wherever possible, should be avoided, since skin abrasions represent the most likely portal of entry to the body. Where the use of sharps is unavoidable, these instruments shall be placed in separate biological disposal receptacles and sterilized before disposal.
- (i) Tissue cultures infected with human-infectious or potentially human-infectious viruses shall be kept in specially dedicated incubators.
- (j) Likewise, frozen stocks of human-infectious or potentially human-infectious viruses should be kept in specially designated and clearly marked liquid nitrogen

tanks and freezers. Laboratory personnel who leave the laboratory for new employment shall ensure that these materials are either discarded or entrusted to another worker. A central register shall be maintained which includes a record of stored cell lines and human-infectious viruses. Principal Investigators are responsible for providing information for the register and maintaining a record of the viruses and infected cell lines used in their laboratories. The maintenance of a central register for the institution/ company/organisation is the responsibility of the IBC.

- (k) Great care shall be taken to decontaminate spills immediately. The correct disinfectant to use in any given situation depends on the organism being handled and is the responsibility of the Principal Investigator in charge of the work to select an appropriate disinfectant. After each session, work surfaces shall be wiped down with an appropriate disinfectant. Where a biosafety cabinet/laminar flow hood has been used for handling amphotropic retroviruses, subsequent use with non-amphotropic viruses can be undertaken following decontamination with an appropriate disinfectant and ultraviolet decontamination.
- (l) Only trained individuals shall be permitted to handle human-infectious recombinant viruses. It is the responsibility of the Principal Investigator to ensure the proper training of personnel in consultation with the IBC.
- (m) Under no circumstances should investigators be infecting cultures of their own cells, or of their immediate relatives, or those of other members of the laboratory.
- (n) Before beginning work with human-infectious (genetically manipulated) viruses where vaccination with the corresponding virus is regarded as an effective means of preventing subsequent infection (e.g. vaccinia, hepatitis), investigators shall be vaccinated.

5. Infection of animals with recombinant viruses

- (a) Infectious animal viruses unable to infect human cells

Viruses of this group are not considered hazardous and accordingly good animal handling practices are appropriate. Infected animals shall be kept in separate cages and be held in the biohazard room separate from non-infected animals. Infected animals should be clearly marked. If possible, the use of micro-isolators should be encouraged to prevent cross-infection. Precautions should be taken to avoid animals escaping and coming into contact with other animals. All waste generated from animals of this group shall be autoclaved before disposal.

- (b) Viruses with the capacity to infect human cells

Animals infected with human-infectious viruses shall be kept in a separate cage which is clearly labelled as containing the particular virus in question. They should be kept in a separate biohazard room from non-infected animals. The main risk is to the handler who shall take great care to avoid being scratched, bitten or exposed to aerosols. Gloves, face mask and protective clothings must be worn to avoid direct contact with tissue and body fluids. Work place should be covered with protective paper which shall be changed regularly. Only trained staff shall handle these infected animals under the supervision of the Principal Investigator. Precautions should be taken to avoid animals escaping and coming into contact with other animals. All waste should be autoclaved prior to disposal.

6. Gene therapy

In projects where viruses are being used to deliver genes to human subjects (gene therapy), the approval from the relevant institutional ethics committees and the Medical Clinical Research Committee (Ministry of Health) should be obtained, unless the therapy has already been established by the MOH as an approved treatment. A separate guideline governs the control of virus production and safety testing procedures in gene therapy experiments.

APPENDIX 6: PROCEDURES FOR WORK WITH HAZARDOUS FRAGMENTS OF DNA

Note for Category B (iv), Section 4.2

When working with isolated DNA molecules or amplifying DNA molecules using techniques such as the polymerase chain reaction (PCR), there are some cases where caution is warranted. Some degree of risk may exist and the extent of this is uncertain.

Such cases include:

1. DNA which encodes an active oncogene product or tumour suppressor gene product, particularly when this is associated with a gene promoter with high activity in human cells. DNA containing more than one active oncogene is associated with increased risk.
2. DNA encoding growth factors, their receptors or other substances that might directly or indirectly alter the growth patterns of human cells.
3. DNA or RNA representing complete viral genomes or fragments with the potential to regenerate live virus. Complete genomes for HIV or papilloma viruses, for example, warrant careful handling.

There is some risk that such molecules could enter the cells of the operator, the principal routes of entry being through breaks in the skin. It is therefore recommended that work of this type be carried out using gloves in order to avoid skin contact. Special care shall be taken when using needles or other sharp instruments.

Precautions for handling genetically manipulated viruses with the capacity to infect human cells are described in Appendix 5.

APPENDIX 7: REQUIREMENTS FOR GENETIC MODIFICATION BIOASFEY LEVEL 2 (GM-BSL2)

The requirements listed here for GM-BSL2 containment emphasise the procedures to be observed by the laboratory worker to ensure a basic level of laboratory safety. There are also some architectural requirements relating to laboratory design and equipment.

The requirements for Biosafety Level 2 in the Laboratory Biosafety Manual (3rd Ed, WHO) shall apply for GM-BSL2. In addition, the following requirements must be included.

Laboratory Procedures

Many of the laboratory procedures detailed below are those that would be considered essential minimum practices in any microbiological laboratory.

1. Storage of food or drink in the laboratory or any storage unit containing genetically manipulated DNA (e.g. refrigerator) is prohibited.
2. Equipment used for handling cultures or contaminated material which is not readily steam sterilised shall be chemically disinfected after use.
3. All technical procedures shall be performed in a way that minimises the creation of aerosols. In particular, operations such as sonication or vortexing which may generate aerosols are to be done in a biological safety cabinet. A period of at least five minutes shall be allowed for aerosols to settle before opening homogeniser or sonicator containers in a biological safety cabinet.

Essential Equipment

Each laboratory shall be equipped with basin mixers for hand washing, preferably foot-operated, elbow-operated or electronically operated. Emergency drench showers and eyewash stations shall be provided.

Laboratory Signs

The laboratory shall be labelled with adhesive signs:

- on the door, with a sign designating the level of containment;
- in the laboratory, with a sign giving procedures required for work at this level of containment.

The signs for laboratories shall be put up only after inspection by the relevant regulatory agency for all higher levels of containment.

Constant Temperature Rooms

Walk-in warm rooms and cold rooms used for incubation or storage of genetically manipulated material may be certified as GM-BSL2 facilities even if they do not contain sinks or coat hooks. Work in such rooms shall otherwise follow all the procedures required for GM-BSL2 laboratories.

APPENDIX 8: REQUIREMENTS FOR GENETIC MODIFICATION BIOSAFETY LEVEL 3 (GM-BSL3)

GM-BSL 3 and 4 facilities must be approved by the relevant regulatory agency before commencement of work. The requirements for Biosafety Level 3 in the Laboratory Biosafety Manual (3rd Ed, WHO) shall apply for GM-BSL3.

Essentially, work in laboratories at GM-BSL3 level shall conform with the procedures set out for GM-BSL2 laboratories, with the following additional requirements:

Laboratory Procedures

1. All aerosol-producing equipment such as that for sonication and vortexing shall be kept and used in the biosafety cabinet.
2. The biosafety cabinet and/or the laboratory shall be decontaminated with formaldehyde gas after major spills of contaminated material. Records of such spills and any other accidents must be kept, detailing date, time and persons involved with the cleaning up measures taken. Such records must be available for inspection at all times.
3. No other work is to be done simultaneously with work requiring GM-BSL3 containment.
4. While work is in progress a sign on the door shall indicate the level of containment required for that work.
5. Protective clothing shall not be worn outside the laboratory; it shall be transported to the decontamination area in sealed bags or boxes. Boxes shall have provision for penetration of steam during autoclaving.
6. No one may enter the laboratory for cleaning, servicing of equipment, repairs or other activities unless the principal investigator or the biosafety officer has been informed and laboratory surfaces have been disinfected.
7. Where a pressure steam steriliser (autoclave) is not available within the laboratory, laboratory wastes shall be bagged and placed in an unbreakable container with a secured lid for transport to the pressure steam steriliser. The surface of the container should be decontaminated with a suitable disinfectant. Wastes shall not be stored outside the facility before they are sterilised.

Laboratory Planning and Construction

8. The laboratory shall not be located adjacent to, nor open onto, corridors used by the general public.

Decontamination

9. Provision shall be made to decontaminate the biological safety cabinet(s) and the room independently with formaldehyde gas, and for the gas to be purged safely to atmosphere upon completion of the procedure.
10. Decontamination of the safety cabinet(s) shall be performed in accordance with the requirements of established International Standards (see Appendix 1) and will

require the provision of a front cover plate and exhaust duct adaptor to fit the particular cabinet.

Pest Control Program

11. A pest control program against insects, rodents, birds, etc. shall be instituted.

Signs

12. The laboratory shall be labelled on the door and wall with GM-BSL3 signs after approval by the relevant regulatory agency.

APPENDIX 9: REQUIREMENTS FOR GENETIC MODIFICATION BIOSAFETY LEVEL 4 (GM-BSL4)

GM-BSL 3 and 4 facilities must be approved by the relevant regulatory agency before commencement of work. The requirements for Biosafety Level 4 in the Laboratory Biosafety Manual (3rd Ed, WHO) and the WHO Laboratory Biosafety Manual for Laboratories and Production Facilities in the Biomedical Sciences Industry shall apply for GM-BSL4.

Essentially, work in laboratories at GM-BSL4 level shall conform with the procedures set out for GM-BSL3 laboratories, with the following additional requirements:

Laboratory Procedures

1. All street clothing, including underwear, shall be removed and retained in the outer clothing change room. Complete laboratory clothing, including shoes, shall be provided by the organisation and shall be used by all personnel entering the facility. When leaving the facility, personnel shall remove their laboratory clothing and store or discard it in the inner change room before showering.
2. Personnel entering or leaving the laboratory shall indicate, either manually or electronically, the time of each exit and entry.
3. There should always be at least two workers present (or, if only one, a second person located at an observation window or in telecommunication or video surveillance) to assist in case of an emergency.
4. The autoclave and fumigation chamber shall be decontaminated after each exposure to the laboratory environment.
5. A primary container holding viable or intact biological material shall be opened only in a flexible film isolator or a maximum containment laboratory. Containers may be opened in other laboratories only if the biological material has been rendered non-infectious or non-toxic, and the space in the primary and secondary containers has been decontaminated.

Laboratory Construction

6. A telephone or other means of outside communication shall be provided inside the laboratory unit.
7. Walls, floors and ceilings of the facility shall be constructed in such a manner as to form a sealed internal shell which facilitates easy fumigation. The internal surfaces of the shell shall be resistant to liquids and chemicals used in the facility and shall facilitate easy cleaning and decontamination. All apertures in the structures and surfaces shall be sealed to prevent vermin or insects from entering the area. Any windows shall be resistant to breakage.
8. A pass-through dunk tank, fumigation chamber or equivalent decontamination equipment shall be provided, so that materials and equipment that cannot be decontaminated in the autoclave can be rendered safe for removal from the facility.
9. A shatter-resistant observation window, or suitable alternative monitoring facilities, shall be installed so that laboratory occupants can be observed from outside the laboratory.

10. An automatically-starting emergency power source, emergency lighting and communication system shall be provided.

Signs

11. The laboratory shall be labelled on the door and wall with GM-BSL4 signs after approval by the relevant regulatory agency.

APPENDIX 10: REQUIREMENTS FOR PLANT HOUSE BIOSAFETY LEVEL 2 (GP-BSL2)

The following standard of plant house and operating procedures (GP-BSL2) is regarded as a suitable minimum for genetic manipulation work with whole plants which falls under Category B in Section 3. Most work with plants will be adequately contained in this standard of plant house.

Plant house work which falls under Category A will require at least GP-BSL2 level of containment, and additional operating procedures and/or a higher standard of construction may be recommended.

Construction

1. The plant house shall have a floor of concrete, or some other substance approved by IBC. Any openings in the walls or roof (e.g. windows, vents, and air supply and exhaust inlets and outlets) shall be screened with fine screens (thirty –gauge 30/32 mesh wire gauze). The drainage exits shall be designed to avoid entry of invertebrates, rodents and insects. Transparent sections of the plant house shall be made of impact-resistant material selected to maintain the integrity of the structure during all foreseeable impact events, including windstorm, and impacts from golf balls, stones picked up by grass mowers, hailstones and the like. If ordinary glass is used, a protective screen shall be fitted.
2. Entrances to the plant house shall be posted with a sign identifying the type of plant house and listing the procedures applicable, including emergency and maintenance procedures.
3. If the plant house is free-standing, it shall have an anteroom for entry and exit. The anteroom shall be fitted with a sticky pest strip or automatic insecticide aerosol device designed to kill arthropods which gain entry. An anteroom is not necessary if the plant house connects directly with a certified small or large scale containment facility.
4. A wash-basin shall be provided within the plant house adjacent to the entry door. Effluent should be disinfected. Where a laboratory is directly connected to the plant house, the basin may be in the laboratory.

Operating Procedures

5. The plant house shall be inspected regularly to ensure that its containment features are intact. Screens, filters and the like shall be cleaned regularly (in accord with manufacturer's specifications when provided).
6. All doors to the plant house shall be locked for the duration of the work except for those periods when personnel are actually working inside it.
7. Hands shall be washed with soap and water before leaving the plant house.
8. Only persons authorised by the IBC are to enter the plant house. All such persons shall be trained to follow normal plant house routines as well as these operating procedures.

9. All plants in the plant house shall be treated as containing genetically manipulated DNA. Work in the plant house other than that involving genetic manipulation shall be discouraged.
10. Operations which may generate aerosols shall be done in a biological safety cabinet as specified for BSL2 laboratory containment.
11. Plants and tissues taken into or out of the plant house shall be carried in closed containers. Waste plants, tissues, soil, soil substitutes and the containers shall be sterilised.
12. Living plants or tissues shall not be taken from the plant house except to a containment laboratory or, with the approval of the IBC, when they are being transferred to another organisation.
13. If the work permits, plants shall be sprayed regularly with a systemic insecticide. The plant house shall be sprayed or fumigated to kill other arthropods (especially mites) at regular intervals, and at the end of each series of experiments. The organisation shall have an effective insect and rodent control program.
14. The experimental materials shall be inspected regularly for signs of arthropod infestation. The inspection regimen shall pay particular attention to mites as they would not normally be excluded by the window and vent screens.

APPENDIX 11: REQUIREMENTS FOR PLANT HOUSE BIOSAFETY LEVEL 3 (GP-BSL2)

Note on plant houses with higher biosafety containment than GP-BSL2

GP-BSL 3 and 4 facilities must be approved by the relevant regulatory agency before commencement of work. Certain experiments may warrant the use of plant houses with additional containment features and operating procedures higher than those of GP-BSL2 (see Appendix 10). Each project will be considered on an individual basis, and additional operating procedures with a GP-BSL2 facility rather than GP-BSL3 may be recommended. The following requirements are recommended for institutions which may be planning to build plant houses providing containment greater than GP-BSL2.

The attached notes are provided so that IBCs can identify some of the requirements which need to be incorporated into the design of BSL3 containment facilities.

GP-BSL3 door signs will be provided after AVA's biosecurity inspection and certification.

The GP-BSL3 standard plant house and operating procedures shall meet all the requirements of the GM-BSL2 standard as well as those listed below. Note that references to plants or plant tissues in GP-BSL2 should be regarded as referring to all organisms when GP-BSL3 standards are being considered.

Construction

1. All structural joints in the plant house, including transparent sections, shall be fully sealed using an elastomeric sealant.
2. If the plant house is free-standing it shall have an anteroom for entry and exit. The innermost door shall have a door -closing device fitted. The anteroom shall be fitted with a sticky pest strip or automatic insecticide aerosol device designed to kill arthropods which gain entry. An anteroom is not necessary if the plant house connects directly with a certified small or large scale containment facility.

Operating Procedures

3. Personnel shall decontaminate their hands by washing with soap and warm water in the wash-basin provided on entering and leaving the plant house. When entering, personnel shall put on overshoes, covering clothes (e.g. gown/boiler suit) and a hat in the anteroom. These garments shall be removed on leaving the plant house and kept in the anteroom (or laboratory) between uses. They shall be laundered regularly.
4. Materials and equipment taken into or out of the plant house shall be treated by a technique demonstrated to be effective in destroying or removing all stages of the life-cycle of arthropods. This requirement applies to soil substitutes and where feasible to soil. Soil substitutes which can be readily decontaminated shall be used whenever possible. Use of soil is discouraged.

APPENDIX 12: PROCEDURES FOR EXPERIMENTS INVOLVING TRANSGENIC ANIMALS

Introduction

Genetic manipulation work which involves the use of animals includes:

- introduction of DNA into the fertilized oocyte or zygote or early embryo, or which may be carried out in or involve whole animals;
- introduction of a fragment of the whole genome or a virus into an embryo to produce a transgenic animal secreting infectious viral particles;
- use of genetically modified micro-organisms to infect animals.

All investigators and IBCs responsible for genetic manipulation work which involves transgenic animals should be familiar with the requirements of the NACLAR Guidelines on the Care and Use of Animals for Scientific Purposes and laboratory biosafety.

Procedures

(A) For Principal Investigators

Investigators planning work which will fall under Category B (i.e., production of transgenic animals) and which will not fall under Category A nor involve planned releases of genetically manipulated organisms should:

1. Complete a GMAC 'Proposal Form for Assessment of Genetic Manipulation Work'.
2. In addition, attach a description addressing each of the points listed in Appendix 13 (1- 8).
3. Submit the proposal to their IBC.
4. Do not initiate work involving these animals until IBC approval has been received.

(B) For Institutional Biosafety Committees

Upon receipt of a proposal for work with transgenic animals, the IBC should:

1. Assess the information provided in the proposal form and the description of the animal facilities. Points to consider in the design of facilities are laid out in Appendix 13.
2. Inspect the proposed facilities.
3. Either
 - (i) approve the proposed protocol and facilities, or
 - (ii) recommend additional precautions and give approval when these have been implemented.
4. Attach a copy of the terms of the approval if IBC approval is qualified in any way, or if additional conditions are required, and provide this advice to the investigator.

5. Ensure that the experimental protocol complies with standard requirements for experiments involving animals.
6. Notify GMAC of the IBC certification of the transgenic-animal holding facilities, and send a copy of the assessment and proposal forms, including IBC advice to relevant regulatory authorities for notification (if necessary).

APPENDIX 13: DESIGN OF ANIMAL FACILITIES

Introduction

Facilities for laboratory animals used for studies for genetic manipulation work of either an infectious or non-infectious nature should be physically separated from other activities such as animal production and animal quarantine.

Animals under experiment may be either small laboratory animals (e.g., mice, rabbits) or large domestic animals (e.g., pigs, sheep, cattle). The requirements for the housing and maintenance of the animals may differ in scale as a result, but the microbiological safety principles are similar.

Housing of transgenic animals

The particular point of concern with transgenic animals is that the result of any insertion of novel genetic material into the genome of an animal is unpredictable, both in terms of the expression of the new genetic material and regarding the effect of this material on neighbouring genes. Furthermore, any escape of the novel genetic material through either the escape of transgenic animals or through their interbreeding with wild or uncontained animals is potentially irreversible.

The containment for transgenic animals varies in different experiment systems. The nature of the containment required depend on the type of animal, the stages in the animals' life cycle that will be involved in the work, the number to be used, and the nature of the genetic manipulation.

Housing of infectious animals

Points to consider for the housing of animals in which infectious agents have been used, including transgenic animals produced by infectious agents.

1. The animal housing must comply with the NAELAR guidelines on the care and use of animals for scientific purposes.
2. Laboratory animal facilities are an extension of the laboratory and may be integral to, and inseparable from, the laboratory.
3. As a general principle, the biological and physical containment recommended for working with infectious agents in vivo and in vitro are comparable.
4. The physical containment levels for work with infectious genetically manipulated animals follow the animal containment levels GA-BSL2 or GA-BSL3 as appropriate for the pathogen. Requirements for GA-BSL2 and GA-BSL3 animal containment facilities are set out in Appendices 14 and 15.

Points to consider

This section lists some points to consider in the design of facilities for genetic manipulation work involving the production of transgenic animals.

The containment required for transgenic animals varies among different experimental systems. The following points govern the nature of containment involved:

Details of Animal Involved

1. Class, genus and species of animal (if transgenic aquatic vertebrates are involved see Appendix 18)
2. Stages in life cycle that will be involved (embryo only to full maturity and reproduction).
3. Approximate number of animals involved at any one time.

Type of Work

4. Whether the work will involve:
 - (a) modification of germline cells;
 - (b) modification of somatic cells;
 - (c) intention to breed;
 - (d) use of genetically modified micro-organisms to infect animals;
 - (e) infectious agents used to produce transgenic animals

Details of the Genetic Manipulation

5. Depending on the type of work, the following points should be considered before deciding upon the level of animal containment (GA-BSL2 & GA-BSL3):
 - (i) nature of DNA to be inserted (origin and characterisation of DNA, references to published work, reference numbers of small-scale proposals covering preparation of the DNA);
 - (ii) method to be used for introducing the DNA (including details of the construction of any vector to be used, references to published work or reference numbers of small-scale proposals covering preparation of the vector, and containment level recommended for work with the vector);
 - (iii) likelihood of the technique to be used for introducing the DNA resulting in the animal bearing an agent potentially transmissible to animals in the same facility or to humans, and the possible routes of transmission;
 - (iv) reproductive capability of the genetically manipulated animal - is the inserted DNA likely to be heritable?

Other Work Being Performed in the Same Holding Facility

6. Are there any other animals being housed in the same facility? If so,
- (i) how many animals are being housed?
 - (ii) are they all the same species and strain? (what other species are involved?)
 - (iii) what is the nature of any experimental work being carried out on the other animals (for example, infectious-disease work, or genetic manipulation work)? Is it likely that these experiments will interfere with the proposed work?
 - (iv) what arrangements are there to ensure that the other animals are kept separate from the transgenic animals?
 - (v) what procedures are in place for the identification of, and accounting for, individual animals?

Disposal of Animals

7. Bearing in mind the points to consider and the information provided above, consider what special precautions are needed, in addition to standard animal-house practices and provisions set out in Appendix 19 of the Guidelines, regarding the disposal of animals.

Transport of Animals

8. If it is necessary to transport the animals alive from the holding facility to the laboratory or another facility, consider what precautions are needed to be taken in addition to the provisions set out in Section 6.2 of the Guidelines.

APPENDIX 14: REQUIREMENTS FOR GENETIC MODIFICATION ANIMAL BIOSAFETY LEVEL 2 (GA-BSL2)

Personnel must receive training in the handling of the animals to be used and an appropriate standard of work supervision must be maintained. The person responsible for the animal experiment must ensure that all those having contact with the animals and waste materials are familiar with the code of practice and are aware of any other precautions and procedures that may be required.

Adherence to the biosafety guidelines does not exempt researchers from practising responsible laboratory animal care and maintain basic welfare standards (useful references: Guidelines for the Care and Use of Animals for Scientific Purposes, NACLAR 2004; Guide to the Care and Use of Experimental Animals, Canadian Council on Animal Care). Researchers should also be aware that they are subject to Singapore laws that legislate against cruelty to animals. This is contained in Part IV of the Animals and Birds Act (Cap. 7).

These requirements are to be used in addition to the applicable requirements for Animal Biosafety Level 2 in the WHO Laboratory Biosafety Manual for Laboratories and Production Facilities in the Biomedical Sciences Industry and requirements for Genetic Modification Biosafety Level 2 in Appendix 7.

Construction

1. Entrance to the animal house must be posted with a sign identifying the type of animals housed and listing applicable procedures, including emergency and maintenance procedures.
2. The animal room must be constructed with impermeable and easily cleanable surfaces. Any openings in the walls or roof (e.g., windows or vents) must be screened with fine screens to prevent the entry and escape of insects and other pests. The doorway and room structure should be rodent-proof. Drainage exits in the floor should always contain water or disinfectant in the trap. Drain traps must be regularly cleaned and disinfected.
3. If the animal house is separated from the containment laboratories, an anteroom in which protective clothing and footwear can be stored is required.
4. An autoclave and/or incinerator for the sterilisation of waste materials must be available on site.

Operating Procedures

5. The animal facility must be inspected regularly by the IBC to ensure that its containment features are intact. Only people authorized by the IBC can enter the animal house. All such people should be trained in normal animal-house procedures as well as these operating procedures. A biosafety manual is prepared or adopted. Personnel are advised of special hazards, and are required to read and to follow instructions on practices and procedures. A record book should be maintained to provide an up-to-date inventory of the procedures performed.
6. Work surfaces are to be decontaminated after use and after any spill of viable material. Eating, drinking, smoking and the storage of food for human use is not

permitted in animal rooms. Personnel should wash their hands after handling cultures and animals and before leaving the animal room.

7. Suitable protective clothing, gloves and footwear should be worn. It is further recommended that such footwear and clothing not be worn in other areas. Protection against inhalation of aerosols, scratches or bites should be considered.
8. Bedding material and waste from animal cages or pens used to house animals infected with genetically modified organisms should be removed in such a manner as to minimise the creation of aerosols. This material should be autoclaved before removal from the animal containment unit.
9. Effective disinfectants must be available for immediate use.
10. Special attention should be paid to constraining animals during experiments. The prevention of their escape must be ensured.
11. Animals or animal tissue transported in or out of the animal house must be carried in closed containers. Animals involved in genetic manipulation experiments are not to be used for other purposes or to provide tissue for other purposes.
12. Cultures, tissues, or specimens of body fluids are placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping. Live animals or animal tissue taken from the animal house must only go to another containment facility or be transferred to another organisation which has suitable animal containment facilities. (See Section 5, or requirements regarding the transfer of infectious materials.)
13. Carcasses of animals infected with genetically modified organisms must be rendered safe by sterilisation before disposal.
14. Insect and rodent control measures must be taken.
15. All incidents, including animal bites and scratches as well as spills and accidents which result in overt exposures to infectious materials are to be immediately reported to and recorded by the person responsible for the work. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.
16. If genetic manipulation experiments are conducted on invertebrates or aquatic vertebrates, the following additional conditions apply:
 - (i) a mechanism should be provided to ensure that neither the organisms nor their gametes can escape into the supply or discharge system of the rearing container (e.g., tank or aquarium); the top of the rearing container should be covered to avoid escape of organisms and their gametes;
 - (ii) in the case of invertebrates that crawl, jump or fly, manipulated and non-manipulated animals should be kept apart;
 - (iii) measures should be taken to enable escaped invertebrates to be detected and recaptured or destroyed; for ticks and mites, containers should be kept over trays filled with oil;
 - (iv) all experimental cages/pens must be numbered and documented;
 - (v) used culture vessels must be decontaminated before disposal or thoroughly cleaned before reuse;

- (vi) flying or crawling arthropods should be handled on white trays to facilitate the detection of escape;
- (vii) the use of an electric insect-control unit should be considered;
- (viii) the activity of arthropods and the risk of accidental escape can be reduced by chilling.

It should be borne in mind that invertebrates can harbour infectious organisms, for example, viruses in mosquitoes, midges and biting flies in soft ticks, trypanosomes in triatomid bugs, organisms from sewage in mollusca, crustacea or echinodermata. Such invertebrates should be kept at a containment level appropriate to the risk from the human pathogens carried by these invertebrates.

APPENDIX 15: REQUIREMENTS FOR GENETIC MODIFICATION ANIMAL BIOSAFETY LEVEL 3 (GA-BSL3)

GA-BSL 3 and 4 facilities must be approved by the relevant regulatory agency before commencement of work. Personnel must be experienced in the handling of the animals to be used and a high standard of work supervision must be maintained. The person responsible for the animal experiment must ensure that all those having contact with the animals and waste materials are familiar with the code of practice and are aware of any other precautions and procedures that may be required. The requirements for GM-BSL3 laboratories also apply to animal facilities at GA-BSL3 level. In addition, the requirements for GA-BSL2 animal containment facilities shall be complied with in addition to the requirements below.

Adherence to the biosafety guidelines does not exempt researchers from practising responsible laboratory animal care and maintain basic welfare standards (useful references: Guidelines for the Care and Use of Animals for Scientific Purposes, NACLAR 2004; Guide to the Care and Use of Experimental Animals, Canadian Council on Animal Care). Researchers should also be aware that they are subject to Singapore laws that legislate against cruelty to animals. This is contained in Part IV of the Animals and Birds Act (Cap. 7).

These requirements are to be used in addition to the applicable requirements for Animal Biosafety Level 3 in the WHO Laboratory Biosafety Manual for Laboratories and Production Facilities in the Biomedical Sciences Industry and requirements for Genetic Modification Biosafety Level 3 in Appendix 8.

Construction

1. Entrance into GA-BSL3 animal facilities is limited to people aware of the potential hazards. The entrance door should be fitted key but freely accessible from inside. The door must be posted with signs identifying the facility and listing the applicable procedures. The name and telephone number of the animal-facility supervisor or another responsible person should be attached.
2. The GA-BSL3 facility must not be accessible to the general public or open onto a public thoroughfare.
3. The facility shall be constructed so that the finishes on walls, floors, ceilings and benches are smooth, impervious and easily cleanable on all surfaces and are able to withstand all relevant decontaminations.
4. All waste from the animal facility shall be decontaminated by autoclaving prior to disposal. When animals are in the room, floor drains should be sealed with an airtight plug.
5. The animal room should be sealable to permit fumigation. It should be possible to decontaminate the GA-BSL3 area independently with formaldehyde gas and for the gas to be discharged safely to the atmosphere upon completion.
6. The facility must have access to an autoclave either within the GA-BSL3 area or in the animal building.
7. The facility must have a hand basin and foot and elbow operated taps located near the exit.

8. Biological safety cabinets are to be used within the GA-BSL3 facility for containment of aerosols and control of infectious material. The cabinet should exhaust to the outside air through a HEPA filter or to the room air extract system.
9. Infected animals may be housed in isolators, safety cabinets, or containment units.

Operating Procedures

10. No other work is to be performed simultaneously with work requiring GA-BSL3 containment. The GA-BSL3 facility must be inspected at least annually by the IBC to ensure that its containment requirements are intact. Only people authorised by the IBC are to enter the animal facility and they can do so only after they have been advised of the potential hazards and meet any specific requirements (e.g., immunisation). A record should be maintained to provide an up-to-date inventory of the animals present and a chronological record of procedures performed.
11. The protective clothing and footwear must be removed before leaving the facility. Dirty clothing must be decontaminated, preferably autoclaved, before being laundered.
12. Special attention must be paid to containing animals during experiments and to the prevention of their escape.
13. Live animals must not leave the facility. Animal carcasses must be rendered safe by sterilisation before disposal. Animals used in genetic manipulation experiments are not to be used for other purposes or to provide tissue for other purposes.

APPENDIX 16: REQUIREMENTS FOR INSECTARY BIOSAFETY LEVEL 2 (GI-BSL2)

The standards specified in Arthropod Containment Guidelines (American Society of Tropical Medicine and Hygiene) in the construction and operation of a Arthropod Containment Level (ACL)-2 insectary are regarded as suitable minima for genetic manipulation work involving insects which falls under Category B(i) in Section 3 of these Guidelines. These standards may also be applicable for work with insects and/or their pathogens where a higher level of biological containment is usually necessary, provided that only non-flying stages of insects (eggs and larvae), and genetically crippled strains of the pathogen is used, such that transmission is not possible. Genetically manipulated insects (GMI) infected with pathogens shall be housed in a separate facility or in different rooms.

Work with genetically engineered insects, or with insects that contain genetically engineered pathogens, which falls under Category A of these Guidelines will require at least ACL2 level of containment, and additional operating procedures and/or higher standard of construction may be recommended (see Appendix 17).

These materials should be disposed of by waste disposal contractors licensed by NEA and in accordance to NEA guidelines on disposal of such waste.

APPENDIX 17: REQUIREMENTS FOR INSECTARY BIOSAFETY LEVEL 3 (GI-BSL3)

Note on insectaries with higher containment than GI-BSL2

ACL3 facilities must be approved by the relevant regulatory agency before commencement of work. Certain experiments with genetically engineered insects (Category B(i) in Section 3) or with insects containing genetically engineered pathogens (Category A(iii) in Section 3) may warrant the use of insectaries with additional containment features and operating procedures higher than those of ACL2 (see Appendix 16). Each project will be considered on an individual basis, and in many cases additional operating procedures to be used with an ACL2 facility rather than an ACL3 may be recommended. The standards specified in Arthropod Containment Guidelines (American Society of Tropical Medicine and Hygiene) in the construction and operation of a ACL-3 insectary are regarded as suitable minima for genetic manipulation work involving insects which falls under Category A in Section 3 of these Guidelines. The above-mentioned requirements are recommended for institutions which may be planning to build insectaries providing containment greater than ACL2.

The notes in this Appendix are provided so that IBCs can identify some of the requirements which need to be incorporated into the design of ACL3 containment facilities.

The ACL3 standard insectary and operating procedures shall meet all of the requirements in the ACL2 standard (See Appendix 16).

These materials should be disposed of by waste disposal contractors licensed by NEA and in accordance to NEA guidelines on disposal of such waste.

APPENDIX 18: PROCEDURES FOR EXPERIMENTS INVOLVING FISH AND OTHER AQUATIC ORGANISMS

Genetic manipulation work involving the use of fish and other aquatic organisms includes:

- production or use of transgenic aquatic organisms;
- use of genetically modified microorganisms to infect aquatic organisms.

The primary objective in designing containment facilities and procedures for genetic manipulation work involving aquatic organisms is to prevent escape of transgenic aquatic organisms or recombinant infectious agents into natural waterways.

Proposals for such work will be examined by IBC on a case-by-case basis. Investigators submitting proposals for research involving transgenic aquatic organisms shall describe in the proposal the containment facilities to be used and procedures proposed for treatment of waste water from the facility.

Adherence to the biosafety guidelines does not exempt researchers from practising responsible laboratory animal care and maintain basic welfare standards (useful references: Guide for the Care and Use of Laboratory Animals, National Research Council, 1996 and Guide to the Care and Use of Experimental Animals, Canadian Council on Animal Care). Researchers should also be aware that they are subject to Singapore laws that legislate against cruelty to animals. This is contained in Part IV of the Animals and Birds Act (Cap. 7).

The following general guidelines are provided regarding the basic requirements for genetic manipulation work involving aquatic organisms.

General

- The rearing area shall be confined in a secured building and be restricted to authorized personnel only.
- All water shall leave the plant through a common drain.
- All effluent water shall be passed through at least two screens. The size of the screening shall be set to retain the smallest life history stage of the organisms in use. The screens shall be cleared regularly to prevent blockage and overflow.
- Effluent water shall not discharge into a major system containing related fish species.
- The building shall be structurally sound and of sufficient elevation to preclude flooding or unintentional escape of these transgenic organisms.

Work with transgenic aquatic organisms

- To prevent escape of adult aquatic organisms into the sewerage system, a grill or mesh, with an appropriate grid size to prevent passage of adults of the species being used, shall be fitted to outlets used for disposal of tank water.
- Water from tanks containing only adult transgenic aquatic organisms, and not involving use of recombinant infectious agents, may be discarded untreated down the sink, provided that a filtering mechanism to retain adult aquatic organisms is in place.
- Tank water that has any potential to contain embryos, sperm, eggs or larvae of transgenic aquatic organisms shall be treated to ensure inactivation of viable

transgenic material before disposal. The proposed treatment procedures and evidence for their efficacy shall be provided to AVA for case-by-case assessment.

- Since many fish species have sticky eggs which attach firmly to substrates, nets and other equipment used in the tank during spawning shall be sterilised after use. If fish are induced to spawn on the sides of tanks, the tanks shall be decontaminated after use by a procedure of demonstrable efficacy in sterilising eggs and sperm for the species used.

Work with infectious recombinant microorganisms in aquatic organisms

- General practices shall be as required for GM-BSL2 or GM-BSL3 laboratory work depending on the microorganism.
- To prevent escape of adult aquatic organisms into the sewerage system, a grill or mesh, with an appropriate grid size to prevent passage of adults of the species being used, shall be fitted to outlets used for disposal of tank water.
- Tank water used for work with infectious recombinant microorganisms, or tank water that has any potential to contain embryos, sperm, eggs or larvae of infected aquatic organisms, shall be treated to ensure inactivation of viable material before disposal. The proposed treatment procedures shall be provided to AVA for case-by-case assessment.

APPENDIX 19: DISPOSAL

I. Inactivation

Waste contaminated with GMMs should be inactivated prior to discharge or disposal. Inactivation is defined (as applied to treatment of waste prior to disposal) as *'the complete or partial destruction of GMMs so as to ensure that any contact between the GMMs and humans or the environment is limited to an extent commensurate with the risks identified in the risk assessment and to provide a high level of protection for humans and the environment'*. This means that the degree of 'kill' achieved by the inactivation must be related to the risk posed by the GMM. For Category A hazardous GMMs, sterilisation will be required. For instance, physical methods and alkaline hydrolysis can be validated to effectively achieve a complete kill. For Category B and C hazardous GMMs, a lower standard might be acceptable, provided that the risks are sufficiently reduced to a 'safe' level. For instance, chemical disinfection typically gives a 5-log reduction in viability of GMMs.

For Category B and C activities, inactivation may not need to be a separate, discrete step in the procedure. Inactivation may be a consequence of other processing steps or experimental methods. For example, extraction methods frequently involve disruption or lysis steps that will achieve sufficient inactivation of the GMM. Alternatively, collection of waste generated from these low risk activities by licensed waste collectors and sending for incineration without autoclaving are acceptable. Care should be taken, however, to ensure that such methods are consistent and provide a level of inactivation that is adequate from a risk management perspective.

A. Physical inactivation methods

1. Physical methods of inactivation are arguably the most reliable way to achieve a high kill rate. For large-scale operations, inactivation will usually involve heat inactivation of cultures. For other operations, the use of an incinerator (for example to dispose of infected animal carcasses) or rendering procedure might be appropriate or sufficient. However, the use of a validated autoclave cycle remains the most effective means of inactivating GMMs.

2. A range of autoclave cycle parameters are suitable for inactivating microorganisms and a typical cycle would be 121 °C, maintained for 15 minutes. Holding-time may need to be increased for work with particular organisms (for example spore-forming bacteria), large amounts of contaminated material or where steam penetration is inefficient. Users should consult the technical specifications for their particular autoclaves. A higher heat setting is recommended for work with transmissible spongiform encephalopathy agents (TSEs) (134-138 °C). Further information on the handling of TSEs can be found in ACDP/SEAC guidance document *Transmissible spongiform encephalopathy agents: Safe working and the prevention of infection*.

3. An autoclave should be available for all laboratory-scale activities with GMMs and those that involve animals. Therefore, the use of an autoclave should always be an option for these activities and it is recommended that one be used for waste inactivation, either by itself or in combination with chemical methods. Care should be taken, however, to ensure that chemical disinfectants do not damage the autoclave. The autoclave should be serviced regularly and its performance tested frequently.

4. Any inactivation method used should be validated or verified under working conditions.

B. Chemical inactivation methods

1. Chemical inactivation methods are commonly used in laboratory-scale operations to avoid the need to autoclave bulk waste (eg spent media and liquid cultures). Chemical disinfection is an inherently less reliable method of inactivation as there are many factors that can come to bear on the effectiveness of the chosen disinfectant. For example, the presence of organic matter can impede the performance of certain disinfectants. Similarly, the disinfection regime may be compromised if cultures are buffered or proteinaceous. Furthermore, the requirement to validate or verify the effectiveness of the procedure stands, and it can be technically difficult or impractical to adequately remove the disinfectant and screen for viable GMMs. For these reasons it is recommended that, for high-risk activities, chemical methods are not relied upon as a sole means of inactivation but are used in combination with physical methods. However, the sole use of chemicals does represent an acceptable means of inactivation for lower-risk activities.

2. When selecting a disinfectant, its toxicity to humans and the environment should be considered. Appropriate safety precautions should be adopted. Different disinfectants must not be mixed together or used in combination unless the possibility of hazardous reactions or the formation of toxic products has been properly assessed. Appropriate procedures should be used to ensure suitable disinfectants at the correct dilution are available at the point of need. Personnel should be trained in the correct use of disinfectants and in the emergency spillage protocols associated with them.

3. There are advantages in limiting the number of different disinfectants available in the workplace to the minimum necessary, in order to avoid confusion and to reduce costs. Once a disinfectant has been selected, in-use tests should be carried out to monitor both the performance of an individual chemical but also the way in which it is used (for example tests to detect incorrect dilution, old solutions and mixtures of incompatible reagents).

4. Disinfectants and chemical inactivation methods in common use are discussed below, including considerations relating to the characteristics, advantages and disadvantages of each.

II. Disposal of Animals

1. When an animal containing recombinant DNA or a recombinant DNA-derived organism is euthanized or dies, the carcass shall be disposed of to avoid its use as food for human beings or animals unless food use is specifically authorized by an appropriate agency.

2. A permanent record shall be maintained of the experimental use and disposal of each animal or group of animals.

III. Disposal of Plants

1. There is a regulatory requirement that all GMM contaminated materials and waste must be inactivated by a validated means prior to disposal. In plant growth facilities, this may include growing media, pots and tools, as well as plant material and other incidentally contaminated items. Autoclaving will generally provide the best assurance of inactivation, but it may not be appropriate for all contaminated materials. When autoclaving, the equipment should be operated so as to comply with the manufacturers' instructions. For example, small amounts of plant material may be inactivated using 121°C for 15 minutes but appropriate times and temperatures may vary. Larger volumes of waste may necessitate a longer holding time or higher

temperature. The key requirement is that the system is validated to ensure sufficient steam penetration to the centre of the load for the required time period is achieved. Incineration is an appropriate alternative, although the risk assessment should detail the risk management procedures in operation. Where the incinerator is located off site, there is a regulatory requirement that the incinerator premises be registered as a GM centre. For Class 1 GMMs, waste material should be double bagged and placed in a suitable container for transfer to waste management facilities.

2. Since Class 2 GMMs have been identified as being able to infect plants in the environment, the containers used for transporting to the incinerator should be sufficiently robust (see M18). Where small amounts are involved validated containment vessels (eg incinerator bins) may be sufficient. One-way burn bins may also be appropriate, but for larger volumes burn bags contained within wheelie bins are acceptable. Local rules should be used to clearly outline the expected fate of all material within the facility and GM and non-GM material may have to be subject to the same waste inactivation measures unless fully justified in the risk assessment.

3. Inactivation of GMMs in effluents from washbasins and showers might be required in Containment Level 3 facilities, where the risk assessment shows that this is necessary. Where this is required, effluents should be collected in a sump and inactivated, or pass through a 'kill tank'. Given the hazardous nature of the material, the containers used for transporting to the incinerator should be sufficiently robust (see M18). One-way burn bins should be sufficient. Local rules should be used to clearly outline the expected fate of all material within the facility and GM and non-GM material may have to be subject to the same waste inactivation measures unless fully justified in the risk assessment.

IV. Disposal of GMM contaminated waste

(applicable for both contained use and deliberate release activities)

1. The inactivation of waste is another area that causes concern among GM users, as the requirement to inactivate GMMs in contaminated waste under the Contained Use Regulations is interpreted as being more onerous than the steps taken when dealing with normal clinical waste. This is not necessarily the case and GM clinical waste should be dealt with in a pragmatic way.

2. Both the form of contaminated material generated and the procedure for dealing with it should be described in the risk assessment. As there is no formal definition of 'waste' in Contained Use Regulations, the risk assessment should identify all types of material that could be regarded as GMM contaminated.

3. The intention of the Regulations is to limit contact with the environment and people, as opposed to preventing all contact. In practice, there may be a need for specific inactivation procedures to limit contact, although the approach taken should be commensurate with the risk. For example, the needle and syringe used to with draw blood samples following patient treatment can be disposed of as any other contaminated sharps. Used vials containing the inoculated material could be treated with a chemical disinfectant or autoclaved prior to disposal.

4. Dirty laboratory coats should be cleaned using routine procedures. This would normally involve a high temperature wash (eg 65 °C), and as the procedures are considered sufficient to deal with blood-borne viruses or enteric pathogens, they should be sufficient for GMMs used in hospitals. Similarly, surgical equipment should be cleaned in the standard way, which has been proven to be effective at preventing cross-contamination.

5. Unused or excess clinical materials containing viable GMMs may be returned to the trial sponsor, or inactivated on site through disinfection or autoclaving, prior to disposal in the clinical waste stream.

V. Disposal of Genetically Modified Arthropods

Transgenic insects should be destroyed by proven appropriate methods such as autoclaving or boiling prior to disposal. Waste materials generated from the ACL2 or ACL3 that could contain any life stages must be treated to render them non-viable before disposal.

APPENDIX 20: PRECLINICAL RESEARCH INVOLVING HIV-1 BASED LENTIVIRAL VECTORS

Introduction:

- The use of lentiviral vectors for gene delivery has been increasing in preclinical research because these systems have attractive features.
- However, the use of lentiviral vectors may also raise biosafety issues.
- The major risks to be considered for research with HIV-1 based lentiviral vectors are:
 - (i) Potential for generation of replication-competent lentiviral (RCL) particles
 - (ii) Potential for oncogenesis and/or perturbation of cell cycle, as a result of overexpression of the inserted gene or cDNA carried within the lentiviral vector.
- These risks can be mitigated by the nature of the vector system (and its safety features) or exacerbated by the nature of the transgene insert encoded by the vector.

Procedures:

Investigators planning work which involves HIV-1 based lentiviral vectors should:

1. Consider a range of parameters including:
 - (i) the nature of the vector system and the potential for regeneration of replication competent virus from the vector components,
 - (ii) the nature of the transgene insert (e.g., known oncogenes or genes with high oncogenic potential may merit special care)
 - (iii) the vector titer and the total amount of vector,
 - (iv) the inherent biological containment of the animal host, if relevant
2. Carry out complete, case-by-case, risk assessments of the planned experiments based on the parameters listed above and choose the lentiviral vector system that is most suitable in terms of safety and project requirement.
3. Unless the vector system is covered in Appendix 2, submit a GMAC 'Proposal Form for Assessment of Genetic Manipulation Work'. In addition, submit other appropriate institutional forms and additional relevant information to the respective local Institutional Biosafety Committee (IBC), which will advise if additional regulatory application is required.
4. Determine the measures and procedures to be instituted to ensure proper containment, handling, storage, and disposal of infectious materials. Some recommendations are provided in Appendix 5. In addition, the risk assessments and proposed measures and procedures may be subjected to review and approval by the IBC or other regulatory body.

5. With regards to the GMAC evaluation criteria, experiments involving the use of lentiviral vectors (excluding third generation and advanced lentiviral vectors⁶) whose host range includes human cells, and where the lentiviral vectors contain inserted DNA sequence(s) coding for a product known to be oncogenic, or to play a role in the regulation of cell growth, or to be toxic to human cells, could typically be classified under Category A (i.e. Regulated Experiments with Significant Risks). In this case, the experiments using lentiviral vectors should be carried out under Enhanced Biosafety Level 2 conditions (i.e. in Biosafety Level 2 facilities, but using Biosafety Level 3 practices and procedures).
6. Conversely, where the vectors used are not third (or advanced) generation lentiviral vectors, and where they do not contain inserted DNA sequence(s) coding for a product known to be oncogenic, or to play a role in the regulation of cell growth, or to be toxic to human cells, the experiments could typically be classified under Category B (i.e. Notifiable Experiments with Low Risks). In this case, the experiments using lentiviral vectors should be carried out under Biosafety Level 2 containment provided that the amount of virus-containing culture produced is less than 1 liter.
7. Satisfy the requirements of the NACLAR Guidelines on the Care and Use of Animals for Scientific Purposes (if animal work is involved) and ensure that the appropriate animal facilities are available (refer to Appendix 13).

⁶ For 'Advanced lentiviral vector' definition, please refer to the MOH Regulatory Policy Branch – <http://www.biosafety.moh.gov.sg>

APPENDIX 21: GLOSSARY

Note: Words are defined in this Glossary according to the use they have in these Guidelines

ACL	Arthropod Containment Level
<i>Agrobacterium tumefaciens</i>	A bacterium which infects plants and contains a plasmid (<i>q.v.</i>) that can be used to introduce foreign DNA into plant cells.
amphotropic retrovirus	A retrovirus (<i>q.v.</i>) that will grow in the cells from which it was isolated and also in cells from a wide range of other species.
amplify	To increase the number of copies of a gene or DNA sequence
autoclave	A device in which materials are sterilised using steam under high pressure.
AVA	Agri-food and Veterinary Authority of Singapore.
bacterium	A single-celled prokaryotic (<i>q.v.</i>) organism.
bacteriophage	A virus that infects bacteria; also called phage .
baculovirus	A group of viruses that infect insects and can be used as vectors (<i>q.v.</i>) to produce foreign proteins in insect cells.
biological safety cabinet/biosafety cabinet	Specially constructed cabinets which are designed to protect workers and the environment from dangerous agents, especially bacteria and viruses.
BATA	Biological Agents and Toxin Act
cell	The smallest structural unit of living organisms that is able to grow and reproduce independently.
characterised DNA	DNA which has been sequenced and for which there is an understanding of the gene products.
chromosome	A structure in the cell, consisting of DNA and proteins, that carries the organism's genes.
clone	As a noun: a group of genes, cells or organism derived from a common ancestor and genetically identical. As a verb: to generate replicas of DNA sequences or whole cells using genetic manipulation techniques.
conjugative plasmid	A plasmid (<i>q.v.</i>) which codes for its own transfer between bacterial cells by the process of conjugation ('mating').
construct	As a noun: genetically manipulated DNA.

containment	Prevention of the spread of genetically manipulated organisms outside the laboratory. Physical containment is accomplished by the use of special procedures and facilities. Biological containment is accomplished by the use of particular strains of the organism which have a reduced ability to survive or reproduce in the open environment.
containment level	The degree of physical containment provided by a laboratory or facility, which depends on the design of the facility, the equipment installed, and the procedures used. GMAC physical containment levels are numbered from 2 to 4, 4 being the highest level.
defective virus	A virus that is unable to reproduce in its host without the presence of another ('helper') virus.
deliberate release	Intentional release of a genetically modified organism into the open environment.
DNA	Deoxyribonucleic acid, the molecule which carries the genetic information for most organisms; consists of four bases and a sugar-phosphate backbone.
donor	The organism or cell from which DNA is derived for insertion into another organism (the host).
<i>Drosophila</i>	A genus of flies whose genetics has been extensively studied.
embryo-rescue	The process in plant breeding whereby tissue from young embryo plants is excised and propagated <i>in vitro</i> for subsequent growth as differentiated plants.
<i>Escherichia coli</i> (<i>E. coli</i>)	A bacterium that inhabits the intestinal tract of humans (and other animals).
<i>Escherichia coli</i> K12	A strain of <i>E. coli</i> that has been maintained in culture in laboratories for many years. It has lost the ability to colonise the intestinal tract of humans and animals, is well-characterised genetically, and is often used for molecular cloning work.
<i>Escherichia coli</i> B	Another well-characterised laboratory strain of <i>E. coli</i> .
eukaryotic	Belonging to the group of organisms whose cells contain a true nucleus. Eukaryotic organisms include animals, plants and fungi.
expression	Manifestation of a characteristic that is specified by a gene; often used to mean the production of a protein by a gene that has been inserted into a host organism.
fungi	Non-photosynthetic eukaryotic organisms, including moulds, that feed on organic matter.

fusion	Joining of the cell membranes of two cells to create a daughter cell that contains the genetic material from both parent cells.
gamete	A reproductive (egg or sperm) cell.
gene	A hereditary unit of nucleic acid which specifies the structure of a protein or RNA molecule.
gene therapy	The replacement of a defective gene in a person or other animal suffering from a genetic disease.
genetic engineering	See genetic manipulation .
genetic manipulation	A technology used to alter the genetic material of living cells or organisms in order to make them capable of producing new substances or performing new functions.
genome	The total genetic complement of a given organism.
genotype	The genetic make-up of an organism, as distinguished from its physical appearance (the phenotype).
germline cells	Gametes and the cells from which they are derived. The genetic material of germline cells, unlike that of somatic cells (<i>q.v.</i>), can be passed to succeeding generations.
GA-BSL	Biosafety Level for genetic modification of animal
GI-BSL	Biosafety Level for genetic modification of insects
GM-BSL	Biosafety Level for general genetic modification work
GP-BSL	Biosafety Level for genetic modification of plants
GMAC	Genetic Modification Advisory Committee.
GMO	Genetically Modified Organism.
GMM	Genetically Modified Microorganism.
growth factor	A protein that stimulates cell division when it binds to its specific cell-surface receptor.
helper virus	A virus which, when used to infect cells already infected by a defective virus (<i>q.v.</i>), enables the latter to multiply by supplying something the defective virus lacks.
HEPA filter	High efficiency particulate air filter.
HIV	Human immunodeficiency virus (a retrovirus).
host	A cell or organism into which foreign DNA is introduced to enable production of proteins or further quantities of the DNA.

host range	For a virus, the range of species that can be infected by that virus.
host-vector system	Combination of host (<i>q.v.</i>) and the vector (<i>q.v.</i>) used for introducing foreign DNA into the host.
hybridoma	A hybrid cell, used in production of monoclonal antibodies (<i>q.v.</i>), which is produced by fusing an antibody-producing cell (a B lymphocyte) with a tumour cell.
IBC	Institutional Biosafety Committee.
<i>in vitro</i>	Literally 'in glass'; performed in a test tube or other laboratory apparatus.
<i>in vivo</i>	In a living organism.
knockout mouse	A mouse that has been genetically modified by deletion or inactivation of a specific gene.
LD50	The dose of a toxin or infectious agent which will kill half of a population of organisms.
microorganism	An organism that can be seen only with the aid of a microscope.
MOH	Ministry of Health, Singapore
MOM	Ministry of Manpower, Singapore
monoclonal antibody	An antibody that is derived from a single clone (<i>q.v.</i>) of hybridoma (<i>q.v.</i>) cells and recognises only one antigen.
NEA	National Environment Agency, Singapore
oncogene	An activated (modified) cellular gene which causes normal cells to become cancerous.
oocyte	A cell that divides to form the female reproductive cell.
packaging	In the process of virus replication, the assembly of the components of the virus to form the complete virus particle.
pathogen	An organism that causes disease.
PCR	See polymerase chain reaction .
phage	See bacteriophage .
plasmid	A small, self-replicating molecule of DNA which contains a specific origin of replication. Plasmids are often used as cloning vectors (<i>q.v.</i>).

polymerase chain reaction	A technique for generating, <i>in vitro</i> , an increased quantity of a target segment of DNA.
prion	An infectious agent of unknown etiology which causes spongiform encephalopathies of humans and animals.
prokaryotic	Belonging to the group of microorganisms whose DNA is not enclosed within a nuclear membrane.
promoter	A DNA sequence, located in front of a gene, that controls expression of the gene. It is the sequence to which RNA polymerase binds to initiate transcription.
protein	A molecule composed of amino acids.
protoplast	A plant or bacterial cell which has had the outer cell wall removed.
receptor	Cell-surface protein to which molecules such as hormones and growth factors bind to exert their effects on the cell, or to which viruses bind to gain entry to the cell.
recombinant	Organisms, cells, viruses etc. which contain recombinant DNA (<i>q.v.</i>).
recombinant DNA	DNA formed by joining, <i>in vitro</i> , segments of DNA from different organisms.
recombination	The occurrence or production of progeny with combinations of genes other than those that occurred in the parents.
replication	Reproduction.
retroviral vector	A retrovirus (<i>q.v.</i>) which is used to introduce foreign DNA into animal cells, usually by replacing part of the viral genome with the foreign DNA of interest.
retrovirus	A virus that uses the enzyme reverse transcriptase to copy its RNA genome into DNA, which then integrates into the host cell genome.
RNA	Ribonucleic acid, a molecule similar to DNA, whose functions include decoding the instructions for protein synthesis that are carried by the genes; comprises the genetic material of some viruses.
sharps	Sharp laboratory items such as syringe needles, scalpel and razor blades, and broken glass.
shot-gun cloning	The production of a large random collection of cloned fragments of the DNA of an organism, from which genes of interest can later be selected.

somatic cell	Any cell of a multicellular organism other than germline cells (<i>q.v.</i>).
Ti plasmid	A large plasmid of the bacterium <i>Agrobacterium tumefaciens</i> (<i>q.v.</i>) which carries genes for tumour induction in some plants. A disarmed form of the plasmid which lacks the tumour-inducing genes is often used as a vector to introduce foreign DNA into plant cells.
tissue culture	<i>In vitro</i> growth of tissue cells in nutrient medium.
toxin	A poisonous substance, produced mainly by microorganisms, but also by some fungi, plants and animals.
transgenic (organism)	An organism whose cells, including the germline cells, contain foreign DNA; transgenic animals are produced by the insertion of the foreign DNA into the newly fertilised egg or embryo.
tumour suppressor gene	A type of gene in which inactivating mutations contribute (or anti-oncogene) to tumour development.
vector	A self-replicating agent (e.g. plasmid or virus) used to transfer foreign DNA into a host cell.
viroid	A disease-causing agent of plants, which is smaller than a virus and consists of a naked RNA molecule.
virulence	Ability of an organism to cause disease.
virus	A submicroscopic infectious particle, containing genetic material (DNA or RNA) and protein, which can replicate only within the cell of an organism (plant, animal or bacteria).
zygote	The cell produced by the union of the male and female gametes.