Biosafety Guidelines in Genetic Engineering and Biotechnology for Laboratory Work

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Biosafety Guidelines in Genetic Engineering and Biotechnology for Laboratory Work

PREFACE

During the past decade substantial progress and development have been achieved in R&D in biotechnology, especially the use of r-DNA technology or genetic engineering. Such achievements have been obvious in several areas including agriculture, industry, medicine and public health. r-DNA-derived products and research extend to various research institutes, universities and private research laboratories located both in the developing and developed countries. Genetically modified organisms (GMOs) (transgenic plants, animals and microorganisms) have been produced and commercial exploitation achieved after meeting necessary requirements in terms of biosafety under laboratory conditions as well as small-and large-scale field trials under different ecological regimes.

As a rule, an investigation on GMOs is undertaken by competent researchers or a research team, taking into consideration good laboratory practices and the acceptable safety of releasing the GMOs into the environment. Nevertheless, such progress and accomplishment has, at the same time, evoked tremendous concerns among researchers themselves as well as the public at large. Such concerns are centered around the release of transgenic organisms into the open environment, biosafety precaution and preventative measures; the fear that certain transgenic organisms may be harmful or become pathogenic to economic plants, animals and human beings; and the unanticipated virulence of manipulated genes or gene products that may disperse uncontrolled and freely in nature.

At the regional and international levels, a number of countries, developing and developed, have prepared or adopted biosafety guidelines for both laboratory investigation and field applications of R&D attempts involving r-DNA. The main objective is to ensure safety and minimize all the risks which are likely to occur, encountered or subsequently generated beyond expectation. Such guidelines may differ from one country to another however the principles are essentially more or less similar. In many cases, acceptable guidelines in developed countries are used as references, subjected to consideration and then modified or amended to be appropriate and in compliance with the existing related laws and regulations within respective countries. At the international level, efforts have been made by UNIDO, FAO, WHO and other international agencies to prepare biosafety guidelines with contributions from international experts to help assist developing countries to formulate their own biosafety guidelines.

As far as Thailand is concerned, there exists an immediate need for national biosafety guidelines to help foster the development of r-DNA technology in the country. These guidelines are important and extremely essential, not merely for researchers within the country, but also for various cooperative and collaborative ventures between national institutions and overseas research partners interested in laboratory testing or additional field trials of GMOs in Thailand. This is one of many rationales upon which the Biosafety Guidelines in Genetic Engineering and Biotechnology for Laboratory Work and for Field Work and Planned Release were developed by the National Biosafety Committee under the National Center for Genetic Engineering and Biotechnology (BIOTEC) of the

National Science and Technology Development Agency (NSTDA).

The objective of these guidelines, prepared by the Ad Hoc Biosafety Subcommittee, BIOTEC is not to enforce stringent regulations such that they will impair related R&D activities in the development of biotechnology within the country. At the same time, the objective is also not to be too lenient to allow unintentional safety discrepancies and negligence leading to misuse and irresponsibility by certain researchers or laboratories.

The scope of these guidelines embraces all work related to gene manipulation employing r-DNA technology for all purposes including the development of transgenic plants, animals and microorganisms, production of vaccines, commercial and industrial manufacturing of r-DNA derived products, and releases of transgenic materials and products into the environment.

The preliminary draft guidelines have been under preparation by the Ad Hoc Biosafety Subcommittee since January 1991. They are for laboratory work as well as for field work and planned release. After the Thai version of the guidelines became available in 1992 the National Biosafety Committee considered it desirable to have an English version of the guidelines for the obvious purpose of international collaboration.

With suggestions and guidance of the National Biosafety Committee, several research agencies and universities in Thailand have established their own Institutional Biosafety Committee to oversee r-DNA research activities in their respective institutions and to coordinate their activities in close consultation with the National Biosafety Committee.

The National Biosafety Committee also realizes that these guidelines are still far from complete and future amendments and revision are unavoidable. The Committee welcomes all advice, suggestions, comments and criticism from all concerned in order to incorporate them and render the present guidelines more feasible and supportive of the overall development of r-DNA technology in the country.

Banpot Napompeth
Chairman
National Biosafety Committee
BIOTEC

INTRODUCTION

With awareness of possible adverse effects resulting from deliberate release of genetically modified organisms (GMOs) on human health and environment, biosafety guidelines in Thailand were developed and the draft completed in June 1992. The National Center for Genetic Engineering and Biotechnology (BIOTEC) PUBLISHED THE GUIDELINES FOR THE FIRST TIME IN Thai language. Since more and more cooperation in biosafety development has been made in the region and at the global level, English translation of the guidelines was undertaken and published in 1996.

The National Biosafety Committee (NBC) IS responsible for implementing the biosafety guidelines with BIOTEC as its Secretariat. An Institutional Biosafety Committee (IBC) has been established at each research agency to take care of biosafety measures and to coordinate work with NBC. These Guidelines should be useful for all scientific workers, project supervisors and administrators in conducting genetic manipulation work.

The Guidelines consist of two parts; the first one concerns transgenic work in laboratories and the second on field testing. However, both parts have common guidelines as follows:

1. The classification of work relating to GMOs according to level of risk and safety (Chapter 2). There are 3 categories: 1) work bearing no risk, 2) work bearing low risk, and 3) work with high risk. It is quite important to have such classification so that risk management and control could be made in three levels accordingly (details are in appendices 7-14)

2. Institutional arrangement in monitoring and control of risk (Chapter 3). There are three groups of personnel and organizations concerned: 1) Principal Investigator and researchers, 2) Institutional Biosafety Committee (IBC), and 3) National Biosafety Committee (NBC). Chapter 3 gives details on roles and responsibilities of these persons and committees.

Thailand Biosafety Guidelines are considered to be a soft law based on voluntary action. However, one part has been combined into an existing Plant Quarantine Act. In 1994 the Department of Agriculture (DOA), Ministry of Agriculture and Cooperatives, has made a "Ministerial Declaration" under the "Plant Quarantine Act" and that all transgenic plants are prohibited imports into the country, unless permission is granted by the Director General (DG) of DOA and only for experimental purpose. The applicants could obtain information on the importation of transgenic plants and application for field testing from BIOTEC (as NBC Secretariat) at the following address:

The NBC Secretariat
National Center for Genetic Engineering and Biotechnology (BIOTEC)
National Science and Technology Development Agency Building
73/1 Rama VI Road, Bangkok 10400
Thailand
Tel : 66-2-644-8150-4
Fax: 66-2-644-8107

These Guidelines are to be used and followed by all researchers and institutes conducting genetic manipulation work. It is anticipated that the Guidelines will be modified periodically for more effective and efficient implementation. Therefore, any

suggestion for modification and improvement from all agencies involved would be highly appreciated.

Chapter 1. Coverage of Guidelines for Laboratory Work

These Guidelines are applicable for all research work--whether conducted in laboratories of the government, of state enterprises, of independent research institutes or of private companies--involved in the construction and/or propagation of viroids, viruses, cells or or- ganisms, carrying novel genetic material which are either improbable to arise naturally or are potentially detrimental towards public safety and environmental health. (Classification in Chapter 2)

Uncertainty as to whether a particular endeavor rests within the scope of these Guidelines should be promptly addressed by submitting a detailed proposal of the experimental conditions to the Institutional Biosafety Committee (IBC) for endorsement or clearance before any work commences. In the event an IBC has not been properly established, a detailed proposal should be submitted to the National Biosafety Committee (NBC) for con- sideration instead. Researchers, with even the slightest doubt in these matters, are encour- aged to seek IBC guidance.

Considering the extent of regulated work defined, for the purposes of these Guidelines, regulated material shall refer to all genetically modified materials (DNA and RNA preparations, viroids, viruses, cells and organisms, modified or constructed through genetic engi- neering), derivatives thereof and the wastes or by-products of genetic engineering practices (containing viable organisms or otherwise).

Chapter 2. Categories of Laboratory Genetic-Manipulation Work

Classification of Laboratory Genetic Engineering and Biotechnological Work in Accordance with Levels of Risk and Safety:

Category 1

> Exempted Work (requiring minimal direction from the IBC or NBC)

Category 2

> Work bearing low levels of risk towards laboratory personnel, the community or the environment.

Category 3

> Work bearing an appreciable level of risk towards laboratory personnel, the community or the environment; gene therapy work; and work for which the risks have yet to be clearly identified and assessed.

2.1 Category 1 Work

2.1.1 Biosafety

Granted that the risks conveyed by Category 1 work appear minimal, exempted work must nonetheless be undertaken in strict compliance with standard practices for conventional microbiological laboratories. Experiments involving pathogenic organisms should conform to suitable containment and precautionary measures including personnel training and instruction. Laboratory staff should be familiar with all pathogenic

organisms under study and aware of the appropriate safety procedures required.

2.1.2 Liaison with the Institutional Biosafety Committee

All work to be considered for exempt status must initially be referred to the IBC for notification and approval. In the absence of an IBC for the institution supporting the work, the responsible researchers should refer their project proposals directly to the NBC for endorsement. A standard form, to be submitted to the IBC for consideration of project exemption, is outlined in Appendix 5. (Individual IBCs are at liberty to design unique forms as well, following the terms of the standard proposal for request of exempt status.) The biosafety committee reserves final judgment on whether a proposal warrants Category 1 standing in light of the information available. As such, no work which falls under the scope of these Guidelines should be initiated before awarded IBC or NBC approval regardless of the risks, or lack thereof, forecast.

For the work that has already been granted exemption, further corrections and modifications of consequence, to any component of the experimental system, which may place the exempt status in jeopardy, must be referred back to the IBC for deliberation a second time. Another notification form should be submitted to the biosafety committee which will proceed to review the amendments and to judge whether such amendments place the work in a higher-risk category. The NBC will be informed of the proceedings if and once the amendments are authorized by the IBC. Otherwise, the researchers must arrange for experimental conditions to be in harmony with the additional specifications imposed on Category 2 or 3 work.

2.1.3 Experiments characterized as Category 1 include the following:

A. Tests involving organisms that naturally exchange genetic material, provided that the donor and the recipient are of the same species or that the donor species is capable of exchanging genetic material with the recipient species under natural circumstances. A list of such combinations is found in Appendix 2.

B. Work with previously approved host/vector systems (Appendix 3) and in which the donor DNA exhibits all of the following properties:

- is not derived from microorganisms which cause diseases in humans, plants or animals.
- represents or comprises no more than 2/3 of any complete viral genome and is employed in such a manner as to disallow the possible regeneration of live viruses (as opposed to such work wherein the hosts carry the missing segments of viral genomes or whereby regeneration is made possible under the context of ensuing propagation sequences).
- does not code for proteins which regulate the growth of mammalian cells (e.g. product of oncogenes), for cytotoxic proteins, or for toxins, to vertebrates, with an LD50 of less than 100 . (Appendix 4)

C. Fusion of protopl asts among non-pathogenic microorganisms or between plant cells (embryo rescue).

D. Fusion of cells derived from higher animals but which does not result in a viable organism such as in the creation of hybridomas without the use of a viral stimulator (e.g. EBV) for production of monoclonal antibodies.

2.2 Category 2 Work

2.2.1 Biosafety

Category 2 work poses low levels of risk towards laboratory personnel, the community or the environment and necessitates, at the very least, containment level C1. Situations involving whole plants and animals should normally be conducted under PH1 plant glasshouses or C1A animal houses. Certain types of work, however, may call for further security precautions or higher levels of physical containment (e.g. special containment conditions in some animal houses designed for transgenic species) because the DNA concerned, or segments and fragments thereof, may conceal hazards or cause diseases. Such work will be negotiated on a case-by-case basis. Regulations and criteria for the procedures, facility design and containment features at the various biosafety levels are outlined in Appendices 7 through 14.

2.2.2. Liaison with the Institutional Biosafety Committee

The project supervisor or head researcher has the primary responsibility for identifying the nature and locality of potential hazards within the laboratory and for determining additional precautions--supplementing the relevant standards laid down in Appendices 7 through 14--appropriate to the levels of risk and concern in this category of work. The need for these additional procedures and conditions to be in line with, and address the specific risks and concerns discovered, should be of paramount interest. The project proposal culminating from such effort on behalf of the project supervisor must be referred to the IBC for consideration. (A form to be completed is outlined in Appendix 5.) The IBC shall evaluate the proposed ambient working conditions and containment resources and determine the sufficiency of these provisions. Considering the risk assessment results, the IBC may also impose special requirements on the work at hand. Only after obtaining IBC approval may the work begin. The IBC must then forward the proposal, and its assessment thereof, to the NBC for record-keeping, and to further monitor safety conditions through the duration of the work.

2.2.3 Experiments characterized as Category 2 include the following:

A. Work with previously approved host/vector systems (Appendix 3) but in which the genetic material inserted exhibits one or more of the following properties:

- codes for proteins regulating cellular metabolism, growth or division.
- represents a pathogenic determinant.
- represents an uncharacterized DNA or RNA sequence derived from microorganisms which cause diseases in humans, plants or animals.

B. Work with non-approved host/vector systems.

C. Genetic engineering of modified whole plants. Supplementary information form required for submission is outlined in Appendix 5.

D. Altering the genome of oocytes, zygotes or early embryos, through any means, to the extent that a novel organism results.

- Genetic manipulation work subject on live animals (microorganisms notwithstanding) to the extent that a novel organism results.

2.3 Category 3 Work

2.3.1 Biosafety

Category 3 distinction is placed on work which poses a substantial level of risk to laboratory personnel, the community or the environment, gene-therapy work, and work for which the character and degree of the risks are as yet uncertain. With such an array under the umbrella of Category 3, appropriate containment levels are far from rigid and may vary considerably depending on the inherent nature of the experiment and on risk assessment results--C1 levels may be adequate for some types of work whereas other situations may demand higher levels and experienced, adept personnel.

2.3.2 Liaison with the Institutional Biosafety Committee

Similar protocols apply as with Category 2 work, although Category 3 work must be reviewed and the proponents counseled by both the IBC and NBC. The IBC serves, in part, as a conduit for the flow of information between the researchers and the NBC, forwarding proposals, assessments and recommendations. Work is prohibited before consent is granted by the IBC and only after both biosafety committees have inspected and reviewed the project proposals, and advised the relevant authorities on any measures that should be followed up on or freshly instilled.

2.3.3 Experiments characterized as Category 3 include the following:

A. Applications of genes determining pathogenicity in microorganisms other than the approved host microorganisms listed in Appendix 3.

B. Transfer of whole viral genomes, viroids or genetic fragments known to initiate infection in humans, plants or animals. In general, excluded from this subcategory, is work using gene sequences less than 2/3 of any complete viral genome and work involving genetic material lacking components vital to the proper functioning of problem operons, to replication, or to the packaging of new viral particles--all provided that experimental conditions disallow the regeneration of live, infectious viruses.

C. Recombinations between complete viral genomes, viroids and/or complementary fragments thereof determined to be of an infectious or pathogenic nature.

D. Alteration of host infection ranges, virulence or infectivity.

E. Work using viral vectors capable of infecting human cells.

F. Work using microbial hosts or vectors which are human, plant or animal pathogens--with the exception of those listed as approved hosts and vectors under Appendix 3.

G. Applications of genetically manipulated DNA sequences coding for proteins known to regulate cell growth or to be toxic to human cells.

H. Work involving toxin producers including:

- DNA coding for toxins with an LD50 of less than 100 (Appendix 4) or otherwise, exhibiting high levels of gene expression regardless of how low the toxicity of the protein encoded.

- uncharacterized DNA from toxin-producing microorganisms which may contain unfamiliar toxin-determining sequences.

I. Use of defective vector/helper virus combinations which heightens the tendency of regenerating non-defective recombinant viruses.

J. Genetic engineering of animals to secrete or to produce viruses, through the injection of viral genetic material or of whole viral genomes into embryos.

K. Propagation through cloning.

- Gene-therapy work through genetic modification of any nature.

2.4 Miscellaneous Notices:

2.4.1

Experiments which do not fall under the scope of Categories 1,2 and 3 but which nonetheless fall under the coverage of these Guidelines as defined in Chapter 1, shall be treated as Category 3 work by the IBC and NBC. Considering this special provision, researchers attempting such uncharacterized work must adhere to the requirements imposed by Category 3 distinction.

2.4.2

The standing of any research work is liable and subject to change in either direction (from lower to higher risk categories or vice versa) as appropriate, new information or of further procedural revisions implemented subsequent to IBC and NBC notification. Project supervisors must submit new project proposals to the IBC for consideration and recommendation before adopting radical operating procedures or substantially changing any parameter of the work (especially approaches to physical and biological containment) which may introduce novel risks, delimit new biosafety levels or warrant change of classification.

2.4.3

Researchers who wish to restrict access to information of commercial significance (e.g. trade secrets or confidential business reports) provided to the IBC and NBC in project proposals, should mark the relevant sections Commercial-In-Confidence.

2.4.4

Knowing that hazards associated with most genetic manipulation work, researchers are strongly encouraged to pursue only those projects servicing the welfare of the community or the environment, local or global, and to consider whether such fruitful and productive ends may be attained through alternative or conventional means that offer less risk.

Chapter 3. Roles and Responsibilities of Pertinent Authorities

The drafting and implementation of safety precautions in genetic engineering and bio-technological work should be supervised and directed by various committees and individuals, representative of all of the following three authorities: The National Biosafety Com- mittee (NBC), the Institutional Biosafety Committee (IBC) and the Project Supervisor.

The various authorities retain different powers and responsibilities in accordance with their respective affiliations (sections 3.1 through 3.3), yet are bound by the common objectives of enforcing and of preserving the integrity and the intent of national guidelines.

3.1 The National Biosafety Committee

The National Biosafety Committee (NBC) was established on January 22, 1993 and its members were appointed by the Minister for Science, Technology and Environment (Appendix 16) following recommendations from the board of directors for the National Center for Genetic Engineering and Biotechnology (BIOTEC), which recognized both the promise and the risks of research and development in this field of genetic engineering and biotechnology and saw through the need to ensure a standard of safety within the discipline. The BIOTEC, through the NBC Ad Hoc Biosafety Sub-Committee (Appendix 17), prepared national biosafety guidelines for work involving genetically manipulated elements, with the safety of personnel, the community and the environment as a major concern. The NBC carries on and furthers the interests of the BIOTEC, and serves to command and harmonize the direction of genetic manipulation work with the protocols laid down throughout, and with the pretenses underlying these Guidelines.

3.1.1 Authorities and Functions

A. Ensure that ambient conditions surrounding genetic manipulation work reflect and adhere to the specifications of national guidelines for the safety of personnel, the community and the environment exposed to the risks borne by the study.

B. Cooperate with the Customs Department and with other relevant state authorities overseeing the import of live organisms to formulate guidelines for the identification, inspection and regulation of transgenic species, exotic and otherwise.

C. Review the aspects of research methodologies in genetic engineering.

D. Identify, characterize and assess the hazards associated with innovative genetic manipulation techniques or research for which the risks are as yet uncertain.

E. Warn the authorities and individuals who are involved with, or who may be afflicted by genetic manipulation experiments, of potential hazards throughout the conduct of work.

F. Recommend, instruct and lend specialist technical expertise to various research institutions and regulatory agencies in setting up appropriate experimental con-ditions for work with specific regulated material.

G. Facilitate all levels of supervision of genetic manipulation work by establishing

and assisting other regulatory bodies in establishing pertinent codes, disciplines and guidelines for the appraisal of biohazards and the management of biosafeguards.

H. Coordinate efforts to inform and educate the public on biosafety issues and on proposed national policies.

- Forge ties with foreign biosafety committees and relevant agencies overseas to ensure that genetic manipulation practices in Thailand address international biosafety concerns and observe universal codes of conduct.

In addition to the authorities and functions listed, the NBC recognizes that no single authority can foresee all conceivable developments in the domain of genetic engineering and biotechnology and reserves the right to consult with the BIOTEC, the various biosafety committees, state authorities and concerned individuals in amending national biosafety policies and pertinent legislation, to suit the existing needs of this discipline.

The NBC shall see to the constitution of Ad Hoc Sub-Committees as necessary to undertake the various tasks accompanying with the extensive responsibilities of the committee.

3.1.2 Responsibilities to Laboratory Research

To ensure that laboratory genetic manipulation work conforms to the regulations circumscribed within these Guidelines, the NBC must address the following charges:

A. Provide advice and assistance to the IBC on the consideration of Category 3 work and, if necessary, on the consideration of other work bearing various lev- els of risk.

B. Suggest practical alternatives, if any, to high-risk laboratory procedures.

C. Prepare and provide to IBCs, the various notification and assessment forms, biosafety guidelines, related documents and assorted signs for facilities.

D. Alert the various institutions and offices, of state or private, engaged in genetic manipulation work, to new developments in biosafety so as not to expose laboratory personnel, the community or the environment to undue risks.

E. Coordinate efforts between pertinent state agencies and private organizations to maintain safety levels in genetic manipulation facilities and to prepare for bio- logical emergencies.

F. Certify higher-level laboratories, plant glasshouses and animal houses pending inspection. Upon request by the institution, and at the earliest convenience, the NBC will inspect a facility and either issue certification, or recommend additional precautions, if elements of the facility are determined to be inadequate to support the types of risk/hazard accompanying work requiring such strict physical containment.

G. Inspect higher-level laboratories and containment facilities on a regular basis. The NBC reserves the right to inspect laboratories and facilities of containment level C2, PH2, C2A, equivalent or higher, at any time subsequent to certification, without prior notice.

H. Inspect systems, equipment and instruments governing ambient biosafety levels

in genetic manipulation laboratories.

- Protect and restrict access to information of commercial significance, not in the public domain, that researchers have provided in project proposals but wish nonetheless to keep private. On the written proposals submitted, researchers will mark such information, Commercial-In-Confidence.

3.1.3 NBC Contact (postal address)

National Biosafety Committee National Center for Genetic Engineering and Biotechnology (BIOTEC)
The 5th Floor, National Science and Technology Development Agency Building
Rama VI Road, Rajthevee
Bangkok 10400 THAILAND
Telephone: (066)-2-6448150-4
Facsimile: (066)-2-6448107-8

3.2 The Institutional Biosafety Committee and the Biological Safety Officer

Institutions and organizations, state or private, engaged, or with the intent to engage, in the purchase, construction, propagation or field release of genetically modified organisms or components must each arrange for the establishment of an Institutional Biosafety Committee (IBC) to serve as the administrative board on matters of biosafety and on compliance with these Guidelines. To grant the IBC freedom to exercise the full extent of its powers in undertaking all of its functions and responsibilities, the pertinent institutions and organizations must appoint appropriate and capable individuals to the IBC (section 3.2.2) and prepare to support the needs and demands of the committee. In addition to the IBC, institutions and organizations, particularly those engaged in industrial-grade or other large- scale work, are encouraged to recruit a Biological Safety Officer (BSO) to work in conjunction with various biosafety committees.

Small research institutions, which may encounter difficulties in constituting an IBC, may alternatively request non-affiliated IBCs to bear the responsibility for monitoring and supervising the biosafety aspects of their work. Agreements of this nature must be formulated between the parties involved and the NBC must be notified of the proceedings. A representative of the smaller institution requesting assistance must maintain close ties with the now-affiliated IBC or more desirably, serve as an acting or even as an honorary member of the committee.

3.2.1 NBC Certification

For the IBC to receive formal endorsement from the NBC, the pertinent institution must submit to the NBC for review, a completed notification form, detailing the academic and professional history, faculty and qualifications of each member appointed to the committee. Subsequently, copies of a second form to be completed and submitted may be obtained from the NBC Secretariat detailing operations within the institution. On the latter form, the NBC will specifically request the following information:

(for laboratory genetic manipulation work...)

- IBC membership (indicate Chairperson, Secretary and organizational structure).
- designated Biological Safety Officer, where applicable.

- an exhaustive list of current projects supported by the institution (indicate classification and detail results of risk assessment).
- an exhaustive list of approved laboratories owned or supported by the institution (indicate classification and title of work in progress).
- an exhaustive list of the institution s plant glasshouses and animal houses, certified and intended for work with transgenic species (indicate classification and title of work in progress).
- relevant medical history of laboratory personnel contracted for activities under higher-level physical containment.

If requested information is lacking for any reason, the NBC shall return the applicable documents and forms to the institution for specific amendments. On the other hand, the institution may wish and should feel free to provide additional information which may influence certification and should thus be brought to NBC s attention.

3.2.2 IBC Composition

With hindsight, these Guidelines are meant, above all, to simply help and provide a framework for institutions, engaged in genetic manipulation work, to consider issues of risk assessment and biosafety. Inasmuch as the intent of these Guidelines should always be respected, the primary responsibility for maintaining various standards and ensuring biosafety rests with the institutions and the researchers concerned, and should never be wholly dependent upon national guidelines or upon the NBC. The IBC, in particular, represents the most integral element in the domain of biosafety--whether the specifics involve supervising genetic manipulation work, attending to the health of personnel, etc.--and therefore should comprise members of high caliber and considerable experience to assume the functions and responsibilities. In addition, the Chairperson of the IBC should retain a senior position under the pertinent institution to ensure the swift adoption of committee recommendations.

To supervise laboratory genetic manipulation work, the IBC shall comprise no less than five members, with at least a recommended four bearing each one of the following distinctions:

- An individual with the faculties and the resources to evaluate, assess and advise genetic-manipulation work for the institution. Ideally, such an individual should be a respected authority in the particular field of research supported by the institution (e.g. plant genetics, human physiology, virus life-history, etc.).
- An engineer with the necessary expertise and practice to examine and assay the integrity of facilities, instruments and tools governing ambient biosafety conditions.
- A Biological Safety Officer, where applicable.
- An individual, not affiliated with the institution, and representing the interests of the community with regards to biotechnology and biosafety.

Recognizing that biosafety issues evoke many disciplines, the IBC should also consider the prospect of establishing working arrangements with individuals--knowledgeable in such areas as law, economics, social work, ethics, community attitudes, and the

environment-- who can serve as consultants-on-call.

The institution should acknowledge and appreciate the critical role assumed by the supervising IBC and should thus support and grant principal authority on biosafety concerns to the committee--such that the committee may exercise the full extent of its powers in undertaking all of its responsibilities and offer criticism and advice without contest.

3.2.3 Powers and Functions

A. Assist researchers in undertaking risk assessment, organizing training programs and generally, in harmonizing experimental conditions with national guidelines.

B. Determine additional biosafeguards and draft supplementary operating procedures for work supported by the institution, in line with and addressing the specific risks and concerns uncovered.

C. Evaluate the qualifications of researchers involved in biotechnological projects and assess whether each retains a thorough understanding of good microbiological practices necessary for the supervision of students, assistants and junior personnel.

D. Monitor all regulated work under progress within the institution and counsel the proponents on issues of biosafety and on compliance with national guidelines on a regular basis, or as requested. The IBC should set apart time for researchers and for laboratory and field personnel to approach the committee with questions, disputes or concerns.

E. Where appropriate, bridge the gap between the NBC and the research teams, and serve as a throughway for the flow of information, ideas and opinions between the two parties.

- Maintain and update a directory of all personnel engaged in activities at every biosafety level, and instruct new personnel on the correct laboratory and/or field practices, emergency procedures and equipment operations at the relevant level.

- Attend to the health of laboratory and field personnel regularly or as necessary, considering test results from baseline serum samples and medical records.

3.2.4 Responsibilities for Laboratory Research

To ensure that laboratory genetic manipulation work within the institution conforms to the regulations circumscribed within these Guidelines, the IBC must address the following charges:

A. Assess all project proposals referred to the committee, and on the basis of the information provided and the risks forecast, determine under which category of work the proposals fall and whether to endorse the work proposed.

B. Maintain records of approved project proposals for laboratory genetic manipulation work (including notifications for project exemption) and the committee s assessments thereof.

C. Forward copies of all project proposals submitted for IBC notification, and the

committee s assessments thereof, to the NBC for records and information--or for review and recommendation in the case of proposals for Category 3 work.

D. Undertake risk assessment, in cooperation with the research teams as necessary, to determine the appropriate containment and biosafety conditions, operating procedures and emergency safeguards for Category 2 and 3 genetic manipulation work, and for the housing, storage or movement of regulated material.

E. Prepare, in conjunction with the research teams, specific contingency plans after undertaking risk assessments and reviewing project proposals.

F. Suggest practical alternatives, if any, to high-risk laboratory procedures.

G. With particular emphasis on Category 3 work, enforce NBC and committee recommendations, and ensure that NBC and committee comments have been acknowledged and promptly addressed.

H. Inspect and certify, before use in genetic manipulation work, C1 level laboratories, conventional animal houses, PH1 plant glasshouses, and quarantine and medical facilities for infected animals. (The NBC will be responsible for certification of higher-level laboratories and containment facilities.)

- Monitor and assay the containment features of, and the working conditions within all laboratories, plant glasshouses and animal houses supporting the institution s work, to ensure that the various facilities are maintained at the standards and requirements delineated in Appendices 7 through 14.

3.2.5 The Biological Safety Officer (BSO)

Institutions and organizations involved in genetic manipulation work should appoint a Biological Safety Officer to the IBC. Alternatively, institutions affiliated with an IBC yet without the services of a BSO may opt to transfer the responsibility of securing a biosafety officer over to the committee. For larger institutions contracting the services of multiple BSOs, the NBC requires that one representative shall be designated and shall serve as the NBC contact or relations officer. BSOs on leave of absence must arrange for competent replacement to take up the forsaken responsibilities.

To meet the objectives of these Guidelines, BSOs should have considerable experience with pertinent biosafety issues and emergency counter-measures. The BSOs are expected to have undergone rigorous training on biosafeguards in order to participate in the training and instruction of personnel, to review (in conjunction with the IBC, and on a regular basis) operating procedures and biosafety records, and to assay the integrity of containment facilities and safety equipment/utilities.

The BSO and the Chairperson of the IBC shall assume direct advisory positions to the head of the institution on all matters pertaining to risk and biosafety, the health of personnel, contingencies at work and infractions of national guidelines. As with the IBC, the BSO shall set apart time for researchers and for laboratory and field personnel to approach the officer with questions, disputes and concerns.

3.2.6 Personnel Care and Management

Institutions and organizations, contracting personnel for work in genetic engineering and biotechnology must ensure, through the IBC, that all personnel have been instructed on

applicable codes of conduct and are conscious of the risks and hazards involved in their line of work. Personnel should receive supplementary training and instruction on laboratory and/or field procedures, emergency safeguards and equipment operations relevant to their line of work periodically. The IBC, the BSO or the project supervisor may administer tests without prior notice to check up the faculties and the caliber of each individual. No one shall be allowed to work under high-hazard or high-risk situations unless they have consistently exhibited good microbiological practice and a requisite understanding of operational routines.

Whether or not, and what measure of health insurance is provided by the institution remains as a matter of deliberation between the labor organizations concerned and the management. Institutions engaged in microbiological genetic research, however, are strongly encouraged to collect and store baseline serum samples from all personnel at risk at regular intervals for future reference--in the event of contingencies whereby individuals are overtly or unduly exposed to regulated material and fall sick from unusual or unex- plained causes. Institutions which do not effect such practices should institute a program immediately, to be supervised by the IBC, especially where work involves toxic, pathogen- ic or infectious determinants. Provisions for serological monitoring, general health surveil- lance and medical treatment must be given due consideration.

Personnel with questions and concerns regarding any issue of biosafety or operational routines should feel free to approach the BSO or the Chairperson of the IBC, among other authorities.

3.2.7 Accidents and Emergencies

The IBC, in conjunction with the BSO and appropriate divisions of the institution, shall adopt a system for reporting laboratory accidents, occupational hazards and personnel exposures, through to the emergency procedures observed in dealing with such incidents. Additionally, the IBC or BSO should maintain complete records of any subsequent absen- teeism attributed to the contingencies reported. Where found necessary, full-fledged inves- tigations should be launched into these matters.

In the event the Chairperson of the IBC believes any incident (e.g. deliberate attempt to circumvent these Guidelines) to be of gravity, of solemnity or of the potential for major repercussions to the community or the environment, the Chair should present the deliberations of the committee to both the NBC and the head of the institution. The various authorities may then cooperate in implementing further measures to deal with the problems uncovered, if need be.

3.3 The Project Supervisor

The project supervisor or head researcher should possess requisite thorough understanding of the codes, regulations and laws applicable to genetic engineering and biotechnological work and exhibit an appreciation for the biosafety concerns that underlie the need for such provisions.

As the officer-in-charge, much of the responsibilities of the project supervisor rests in the initial stages of originating proposals and obtaining IBC approval, where necessary. For laboratory genetic manipulation work, the project supervisor should assess the nature of the research and determine whether the work proposed falls within the scope of these Guidelines. Uncertainty and doubt should be addressed by submitting a

detailed proposal of the experimental conditions to the IBC for endorsement or clearance before any work is carried out. If work is indeed regulated under these Guidelines, the project supervisor must submit a completed project proposal form (including requests for exempt status) to the supervising IBC for consideration and recommendation, and inform the committee of any notable intents (e.g. plans to import regulated material). Laboratory work may begin after authorization from the IBC. As directed by the IBC, the project supervisor may be required, from time to time, to provide additional details of the research for the various evaluation and monitoring activities of the committee.

The project supervisor should sincerely enforce the provisions and adhere to the intent of these Guidelines through the duration of research work, with special emphasis on the following charges:

A. Submit new project proposals to the IBC for consideration and recommendation before adopting radical operating procedures or substantially changing any parameter of the work (especially approaches to physical and biological containment) which may introduce novel risks, delimit new biosafety levels or warrant change of classification.

B. Establish and maintain working conditions appropriate to the level of biosafety as approved and advised by the IBC--and, in the case of Category 3 work, in accord with the recommendations of the NBC.

C. Ensure that students, junior personnel, co-investigators and other persons entering controlled areas realize the nature and degree of the risks involved and have been properly instructed on applicable codes of conduct.

D. Cooperate closely with the IBC and BSO in carrying out various tests and safety audits, for instance, inspections of containment facilities and personnel examinations.

E. Report all personnel developments, including extended absenteeism, replacments and unusual illnesses, to the IBC.

- Relay to the IBC, details of all contingencies and the emergency procedures instigated to deal with such incidents.

Chapter 4. Special Requirements for Some Laboratory Genetic Manipulation Work

4.1 Work Involving Hazardous Genetic Fragments

(Note for Category 2 work, sub-category A, under section 2.2.3)

Isolating genetic material for laboratory use in host/vector systems may convey various risks and hazards--some unpredictable and most uncharacterized--depending, primarily, upon the nature of the genetic material involved. In particular, the following materials are recognized to bear substantial risks and hazards to laboratory personnel:

- DNA coding for various proteins, known or suspected to directly, or indirectly, regulate cellular metabolism, growth or division (e.g. growth factors/hormones and their receptors).

- Genes encoding lethal or virulent toxins.
- All oncogenes, especially those exhibiting high levels of gene expression or linked to gene promoters with high activity in human cells. For a particular DNA fragment, hazards are multiplied with the number of oncogenes encoded.
- Whole viral genomes. Use of retroviruses carrying oncogene sequences is dealt with under section 4.2 below.
- Fragments of viral genomes which retain the potential to regenerate live viruses.

The principal concern of work with hazardous genetic fragments is that the material involved may penetrate the skin of personnel through cuts and surface wounds or contaminated needles, enter cells and be introduced into tissues. As such, gloves are required, at all times, to avoid skin contact and extreme care must be taken in handling sharp instruments, blades and edges (e.g. needles and scalpels).

4.2 Use of Live Viral Vectors

Many vectors in common use are live viruses, developed to achieve an unprecedented level of efficiency for the transfer of genetic material into cells. Live viral vectors, however, are often associated with a number of risks and hazards, most not fully characterized but all dependent upon the following criteria:

- Host range of the virus.
- Modes of transmission (e.g. air-borne, through body fluids).
- Infectivity and communicability (including, epidemiological considerations).
- Opportunities for repeated rounds of infection.
- Natural tendency of viral genetic material to become inserted into host cells and incorporated into host DNA.
- Nature of foreign genetic material inserted (including, functions of target genes).

Nearly all proposals for use of live viruses as vectors fall into Category 3, and accordingly, must be sanctioned by the NBC before work may begin.

(**Note**: The following considerations and recommendations are meant to apply to the use of retroviral vectors but may be adapted for genetic manipulation work with other classes of live viral vectors as well.)

4.2.1 Retroviral Vectors

The retroviruses developed thus far, have proven to be highly efficient for the transfer of genetic material into a variety of cells. In principle and task, retroviral vectors are no different from conventional eukaryotic vectors in that all provide the fundamental regulatory sequences for the control of introduced gene expression.

The majority of retroviral vectors are *replication-defective* because the target gene(s) substitutes for or displaces a sequence of the genome regulating some important parameter of viral replication or of packaging of viral particles. However, this capability for viral propagation can be restored in cases where the missing genes are provided to

the experimental system by the use of specific non-defective helper viruses. Alternatively, helper functions may be stabley integrated into one of the chromosomes of a variety of packaging cell lines. Such lines will allow for the regeneration of fully infectious retroviruses but which cannot replicate and are not communicable/transmittable. Either way, the types of cells that may serve as hosts (i.e. that may be infected), depend on the particular helper virus adopted, or helper functions afforded by the packaging cell line chosen.

Retroviruses can be classified in a number of ways, but in the interests of these Guidelines, it is helpful to consider retroviruses on the basis of host range, as infectivity is a major concern of general biosafety considerations. For genetic engineering and biotechnology, the following standard definitions should be adopted:

Classes of Retroviruses According to Host Range

Amphotropic viruses -

> The amphotropic viruses will grow in the cells of the species from which they were isolated and also in the cells from a wide range of other species.

Ecotropic viruses -

> The ecotropic viruses will grow in the cells of the species from which they were isolated and to a limited or undetectable level, in the cells of other species.

Xenotropic viruses -

> The xenotropic viruses are endogenous to a species but cannot replicate well in that species, generally because of a receptor block. They tend to have a wide range for replication in the cells of heterologous species.

Source: RNA Tumor Viruses, edited by Robin Weiss, Natalie Teich, Harold Varmus, John Coffin, Cold Spring Harbor, 1982.

As the viruses described all bear appreciable levels of risk and hazard (highly infectious), extreme caution is warranted regarding the nature of the target gene(s) when work ing with retroviral vectors. Where the introduced genetic material is itself hazardous, the risks involved are multiplied. In particular, extreme caution must be observed in handling retroviral vectors, capable of infecting and/or propagating themselves in human cells, which incorporate the following hazardous genetic material:

- known oncogenes of viral or cellular origin.
- genes coding for proteins which regulate, or may alter, the growth patterns of mammalian cells or forming the pattern of traits (e.g. growth factors/hormones and their receptors).
- genes encoding molecules whose activity/expression is modulated by the stimulation of growth factor receptors.
- genes coding for known or suspected toxins (e.g. cytotoxic proteins).

As with hazardous genetic material, the principal concern of work with retroviruses is the possibility of viruses entering cells and tissues through surface wounds and broken skin, or by accident. Most of these risks are directed towards those involved in the

actual manipulations or working in the immediate vicinity, as retroviral particles are extremely labile and short-lived.

Construction of recombinant retroviruses must comply with basic national biosafety guidelines for laboratory genetic manipulation work. Once constructed though, the handling and application of these viruses must likewise proceed in accordance with the relevant supplementary procedures/practices detailed hereinafter (sections 4.2.2 through 4.2.5).

4.2.2 Approach to Non-Human Ecotropic Retroviruses

Non-human ecotropic retroviruses, because of perfectly limited host range, will not grow to any significant level in human cells and are not regarded as dangerous to laboratory personnel. Simply the adoption of good tissue culture practices is enough to ensure a satisfactory laboratory biosafety environment. Nevertheless, it is strongly recommended that various fluids, wastes and by-products from experimental cell cultures and organisms be thoroughly decontaminated/inactivated before disposal, to prevent such ecotropic viruses from fortuitously infecting natural cell lines.

4.2.3 Approach to Defective Retroviruses with Human Cell Host Range

Inherently, retroviruses with human cell host range are amphotropic, xenotropic or human ecotropic viruses. All may bear substantial risks to laboratory personnel if associated with hazardous gene sequences (section 4.2.1). Even where the risks are reduced by the use of defective retroviruses, investigators must strictly abide by good virological and tissue culture practices, with special emphasis on the following precautions:

a. Perform all manipulations and handle all cultures of viable retroviruses in Class II biological safety cabinets or equivalent primary containment units. Only one individual may use a biological safety cabinet at any one time.

b. Handle cultures of amphotropic retroviruses or of cells, infected with amphotropic retroviruses, in double-hulled units (e.g. plates within inverted lids) to trap accidental spills.

c. Store amphotropic viral stock in an appropriately designed and isolated section of the freezer and store ampoules of frozen amphotropic cell lines in a separate section of the liquid nitrogen tank. All such divisions need to be properly labeled/tagged. Laboratory personnel are responsible for the care of amphotropic viral stock and cell lines.

d. Separately incubate tissue cultures infected with amphotropic retroviruses from tissue cultures infected with ecotropic retroviruses.

e. Cover all surface wounds and broken skin (e.g. abrasions, lesions) before entering controlled areas.

f. Take special care to avoid skin contact with retroviruses and contaminated material. Wear cover-alls and rubber gloves whenever operating on retroviruses carrying hazardous genetic material, as described in section 4.2.1

g. Wear molded surgical masks or respirators, as necessary.

h. Keep the use of sharp and pointed instruments (e.g. hypodermic needles, blades,

syringes) to a minimum and take special care to avoid auto-inoculation. If practical alternatives exist, never use sharp instruments on amphotropic retroviruses and cell lines. If not, these instruments must be discarded into separate biological disposal receptacles before sterilization.

i. Use only mechanical pipetting devices; mouth pipetting is prohibited.

j. Decontaminate all pipettes and glassware after use, by autoclaving or using chlorine preparations.

k. Autoclave effluents, wastes and by-products before disposal; exhaust gases from primary containment units and hoods must be treated by high-efficiency filters before release.

l. Wash down contaminated surfaces with chlorine disinfectants and immediately decontaminate spills. Project supervisors have the responsibility for determining the appropriate disinfectant to be used in any given situation, considering the nature of contaminant and of work surface contaminated. As a standard practice, glutaraldehyde disinfectants should be used in Class II biological safety cabinets.

m. Wipe down work surfaces with chlorine or iodide preparations, glutaraldehyde disinfectants or other disinfectants, as suitable, after each session and before ultraviolet sterilization.

Both the project supervisor and the IBC must be actively involved in monitoring biosafety conditions within the laboratory. Before work with genetically modified or recombinant retroviruses, it is critical that a serum sample be kept for future reference and health surveillance, in case of occupational hazards that lead to overt or undue exposures. During the conduct of research, it would be helpful to maintain a central register, providing a chronological inventory of work with, and storage/disposal of, retroviral vectors and cell lines. Above all, and at all times, insurance must be made that only highly trained and proficient personnel operate on infectious retroviruses with human cell host range.

Work with amphotropic retroviruses warrant exceptional caution. Hoods used in manipulations of amphotropic retroviruses must be meticulously wiped down with chlorine disinfectants, and subsequently ultraviolet-sterilized, before use with other non-amphotropic retroviral strains. Where recombinant amphotropic retroviruses are to be propagated in human cells, there is a need to show that cultures are free of HIV and other human retroviruses. Nonetheless, cell cultures from laboratory personnel and their immediate relatives must never be infected under any circumstances, with any class of retrovirus.

4.2.4 Handling of Animals Infected with Non-Human Ecotropic Retroviruses

As non-human ecotropic retroviruses bear negligible hazards towards laboratory personnel, the adoption of good animal handling practices is considered adequate for work with infected animals. As a standard procedure, wastes, secretions and by-products from experimental animals must be thoroughly decontaminated before disposal, to avoid inadvertent infection of wild relatives and natural variants.

Infected and uninfected animals must be kept in separate cages (but may be held in the same room) to minimize the opportunity for repeated rounds of infection.

4.2.5 Handling of Animals Infected with Retroviruses with Human Cell Host Range

The infectious nature of retroviruses, with human cell host range, emphatically need stricter precautions to those precedent. As basic requirements, infected species must be kept in separate, appropriately labeled cages (and apart from uninfected animals) and all wastes, secretions and by-products from experimental animals must be thoroughly decontaminated/inactivated before disposal. In the laboratory, work benches should be layered with protective paper, which needs to be replaced regularly.

On top of good animal handling practices, stringent arrangements must be made to enhance physical containment, preventing escape and spread of infection. Extreme caution must be observed when dealing directly with infected animals to avoid being bitten, clawed or scratched-- in performing operations, gloves and coveralls must be worn to avoid skin contact with animal tissues and body fluids.

In spite of all safeguards, handling animals, infected with retroviruses with human cell host range, still guarantees much risk. Only highly trained and proficient personnel, under supervision of the project supervisor, shall be allowed to operate on infected animals.

4.3 Work with Whole Plants

(Note for Category 2 work, sub-category C, under section 2.2.3)

Recognizing the relative facility with which many plants propagate, disperse and outcross, genetic manipulation work on whole plants requires for submission a *Supplementary Information Form for Laboratory Genetic Manipulation Work on Whole Plants* in addition to the standard project proposal. Supplementary information requested include details of the auto-ecology of relevant noxious weeds and any plans for cultivation of transgenic species. The additional details provided in supplementary information forms will be reviewed by the IBC and shall constitute the framework for committee recommendations regarding containment, disposal and safeguards.

Other special requirements, for the housing of whole plants in genetic manipulation work, are outlined in Appendices 10 through 12.

4.4 Engineering of Transgenic Animals

(Note for Category 2 work, sub-categories D and E , under section 2.2.3)

All genetic manipulation work subject on live animals, fertilized oocytes or early embryos, to the extent that a novel organism results, falls into Category 2. Nevertheless, proposals for such work must be reviewed and approved by the NBC (particularly where work entails the introduction of viral genetic material or whole viral genomes into zygotes and embryos) before official IBC authorization may be issued.

4.4.1 References

The following references are essential to all investigators and IBCs involved in genetic manipulation work with transgenic animals:

- Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 1990 (prepared by the NH&MRC, the Commonwealth Scientific and

Industrial Research Organization, and the Australian Agricultural Council).

- Australian Standards 2243-1991 Safety in Laboratories: Part 3-Microbiology (specifies basic standards for animal houses).
- Procedures for Assessment of the Planned Release of Recombinant DNA Organisms, 1987 (available from the GMAC Secretariat, GPO BOX 2183 Canberra ACT 2601, Australia).

Other key references and recommended reading are listed under Appendix 1.

4.4.2 Special Procedures for IBC Notification

Where proposed work involves the engineering of transgenic animals, the project supervisor must prepare a Supplementary Information Form for the Engineering of Transgenic Animals, addressing the various concerns in the design of animal containment facilities (requirements for the housing of animals in genetic manipulation work are outlined in Appendices 13 and 14). Attach all supplements to the completed Project Proposal Form for Assessment of Laboratory Genetic Manipulation Work and submit to the supervising IBC. This supplementary form will guide the IBC in reviewing the design of animal holding facilities to be used, as appropriate containment measures will vary with the different experimental and biological systems planned or in practice. As always, the project supervisor must ensure that no work shall be undertaken prior to receiving IBC endorsement.

4.4.3 Special Procedures for IBC Assessment

In the consideration of a proposal for the engineering of transgenic animals, the IBC must inspect all animal containment facilities to be used, aside from reviewing the various written forms submitted. Where work raises moral questions and concerns, the IBC should consult with an institutional bioethics committee.

If the IBC endorses the proposal and all animal houses, the committee must immediately forward copies of relevant assessments and the original submissions to the NBC--otherwise, inform the project supervisor of any additional precautions and amendments which must be effected for work to be approved. Only after receipt of NBC recommendations and complete reassessment of proposals, may the IBC give authorization for research work to begin. If final endorsement is conditional upon the adoption of additional provisions, send a copy of the terms of approval--under heading (9) of the *IBC Form for Assessment of a Proposal to carry out Laboratory Genetic Manipulation Work*--to the project supervisor.

4.4.4 Some Points to Consider

Experimental animals may range from small, laboratory species (e.g. fruit flies, mice, rabbits) to large, domesticated species or livestock (e.g. water fowls, pigs, cattle). As such, the scale of housing requirements may vary considerably from one proposal to another, although in principle, microbiological safety considerations would remain similar. On a related note, in case of the considerable scale involved, NBC guidance may be required of work where large numbers of transgenic animals are to be handled together.

The outcome of any introduction of novel genetic material into the genome of animals is never entirely predictable. No matter how precise the method of introduction, the

desired gene expression cannot be guaranteed, and transgenic animals may instead exhibit incidental genetic and phenotypic variability (depending on a number of factors, particularly the stability of incorporated transgenes, their effects on neighboring operons and related changes in biosynthetic and energy demands). In and of itself, the method for introduction of donor DNA can likewise bear undesirable side-effects. Researchers must refer to the IBC, instances where side-effects might include having transgenic animals harbor infectious agents, transmissable to other animals or humans within the same holding facility--and the possible/likely routes of transmission if there is indeed a concern.

Ideally, accommodations for experimental animals in laboratory genetic manipulation work, need to be physically separated from facilities supporting other activities, such as animal nurseries and animal quarantine areas. Where there is to be concurrent use of a transgenic animal house, researchers must adopt suitable precautions to avoid interference with the proposed work.

4.5 Work with Infectious Animals

Infectious genetically manipulated animals--including transgenic animals produced by the introduction of genes from infectious agents, and animals which incorporate genes encoding infectious particles--need special housing considerations addressing the particular risks conveyed. For the most part, physical containment precautions for laboratory work with these animals would follow the general requirements of C1A or C2A animal houses (Appendices 13 and 14 respectively), as appropriate to the level of concern. On occasion, however, higher levels of animal containment is warranted and investigators must refer such instances to the NBC for consultation with the committee regarding special conditions to be adopted. In all cases, as a term of reference, the housing of infectious animals must observe the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes*, NH&MRC/CSIRO/AAC, 1990. Ideally, animal houses must be integrated with the main laboratory to facilitate transport, observation and experimentation.

As a general principle, the biological and physical containment considerations, for this nature of work (*in vivo*), are comparable to those for work with infectious agents *in vitro*.

Chapter 5. Movement of Regulated Material

5.1 Movement of Regulated Material Within or Between Institutions

Extreme care must always be taken in moving regulated material within and between institutions. Genetically modified material must be transported in securely-sealed, double-containment units, each comprising a primary container for holding the organism(s) or culture/preparation, enclosed in a durable, shock-absorbing secondary container which may be readily decontaminated. These units should ideally be placed within sturdy outer shipping containers--soundly packaged and appropriately labeled and addressed to facilitate inspection, to allow for swift delivery to the intended destination and to ensure that relevant authorities are contacted in case of emergencies. Movement of wastes and by-products from genetic engineering practices, require comparable packaging and container specifications.

5.1.1 Transport of genetically modified microorganisms

The essential restriction on the transport of viable genetically modified microorganisms allows for only those arrangements which ensure that transgenic species in transit will not be harmful to the community or the environment if the packaging or container integrity becomes disturbed en route. Species recognized as benign to humans and the environment may be transported within basic packaging and container requirements. Microorganisms, pathogenic, infectious or hazardous to the environment in one way or another, shall only moved provided that the mode of transport offers exceptional and suitable decontamination features.

5.1.2 Transport of transgenic plants and animals

The transport of transgenic plants and animals should be supervised by an ecologist or biologist skilled and with considerable experience in handling transgenic species and in initiating population control programs in the event of unforeseen contingencies. Stringent and selective containment must be adopted as necessary--taking into account, to the greatest extent possible, various contingencies which may be encountered--so as to minimize the potential for escape and to prevent transgenic species from interbreeding freely with and becoming established in wild populations. Proper arrangements should be made to identify and account for individual animals, plants or containers in transit.

As to the transport of transgenic plants, it is recommended that whole plants be netted and deflowered beforehand, and that plants which have set seed not be moved.

5.1.3 IBC Arrangements

The IBC may impose additional security precautions as it sees fit, to address the specific risks and concerns of any transport at hand. Furthermore, the IBC may feel obligated to personally survey and investigate the preparations for transport of transgenic species, to ascertain whether standard requirements and additional precautions, if any, are being attended to.

5.2 Distribution and Receipt of Genetically Manipulated Material

Researchers distributing genetically manipulated material to scientists and institutions, local or abroad, must provide recipients with reviews of the physico-chemical and biological containment measures, safety precautions and any special guidelines for work involving the material circulated. Researchers should also detail the origin of regulated material distributed, to serve as terms of reference for each recipient. In the event of a local beneficiary without previous links to, or background in genetic engineering and biotechnology, the distributor has a further responsibility to ensure that the recipient is made aware of the national guidelines regulating work in this discipline.

Distribution and receipt of genetically manipulated material must be reported beforehand to the appropriate director of the institution for legal purposes.

5.3 Import and Export

Individuals who wish to import viable microorganisms, plants or animals, genetically modified or constructed, must proceed in accordance with the relevant guidelines presented here and are strongly encouraged to consult with the IBC regarding the specifics of their intent. Import of live or whole organisms of another nature is regulated directly by the various orders and enactments presented under Appendix 15.

On the other hand, international postage or export of regulated material must strictly comply with the revised provisions and requirements of *The Non-Infectious* and *The Infectious Perishable Biological Substances Services* as agreed to by the International Postal Union (IPU).

Import and export of pathogens must observe the terms of the *Diseases and Animal Toxins Act of 1982.*

Chapter 6. Sanctions

Scientists who, and institutions which fail to enforce the provisions or adhere to the intent of these Guidelines may be penalized by the withdrawal of applicable or all govern- ment research grants. In addition, non-compliance on the part of private organizations awarded special incentives (e.g. funding from the government or tax incentives) for contributing to biotechnological research and development may result in the withdrawal of said incentives.

Scientists and institutions can be held accountable for all the evident consequences (accidents, medical emergencies and disturbances to the community or the environment) of their failure or neglect to comply with the terms and principles of national biosafety guidelines.

The National Biosafety Committee shall update and inform the Prime Minister on all issues pertaining to the violations of these Guidelines. The Prime Minister reserves the authority to issue public statements on any such issues of infraction, deliberate or otherwise.

Appendix 1

References and Some Related Documents (for recommended / supplementary reading)

1. Guidelines for Small Scale Genetic Manipulation Work. 1993. Genetic Manipulation Advisory Committee, Department of Administrative Services, Australia.

2. Guidelines for Large Scale Genetic Manipulation Work. 1994. GMAC, DAS, Australia.

3. Guidelines for the Planned Release of Genetically Manipulated Organisms. 1993. GMAC, DAS, Australia.

4. Guidelines for the Preparation and Presentation of Applications for General Marketing of Monoclonal Antibodies for Use in Humans. 1988. Department of Health, Housing and Community Services, Australia.

5. Australian Code of Good Manufacturing Practice for Therapeutic Goods. 1990. Therapeutic Goods Administration, DHHCS, Australia.

6. The National Health and Medical Research Council Statement on Human Experimentation and Supplementary Notes. 1992. National Health and Medical Research Council, DHHCS, Australia.

7. Ethical Aspects of Research on Human Gene Therapy: Report to the NHMRC. 1987. NHMRC, DHHCS, Australia.

8. Laboratory Biosafety Guidelines, AIDS Task Force. 1986. NHMRC, DHHCS, Australia.

9. Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. 1990. NHMRC, Commonwealth Scientific and Industrial Research Organization, and the Australian Agricultural Council.

10. Infection Control Guidelines - Acquired Immune Deficiency Syndrome (AIDS) and Related Conditions. 1990. Australian National Council on AIDS, DHHCS, Australia.

11. Biological Safety Cabinets (Class I) for Personnel Protection. 1981. Standards Association of Australia.

12. Laminar Flow Biological Safety Cabinets (Class II) for Personnel and Product Protection. 1985. Standards Association of Australia.

13. Regulatory Control of Veterinary Drugs. 1983. Australian Agricultural and Veterinary Chemicals Council, Department of Primary Industries and Energy, Australia.

14. Requirements for Clearance of Veterinary Chemicals. 1985. AAVCC, DPIE, Australia.

15. Requirements for Clearance of Veterinary Drugs. 1985. AAVCC, DPIE, Australia.

16. Laboratory Containment Facilities for Genetic Manipulation. 1988. Advisory Committee on Genetic Modification, Health and Safety Executive, UK

17. Guidelines for the Categorisation of Genetic Manipulation Experiments. 1988. ACGM, Health and Safety Executive, UK

18. Categorisation of Pathogens According to Hazard and Categories of Containment. Advisory Committee on Dangerous Pathogens, Health and Safety Executive, UK

19. Recombinant DNA Safety Considerations. 1986. Organization for Economic Co-operation and Development (OECD).

20. Guidelines for Research Involving Recombinant DNA Molecules (updated continually). Office of Recombinant DNA Activities, National Institutes of Health, USA.

21. Biosafety in Microbiological and Biomedical Laboratories. 2nd ed. 1989. United States Department of Health and Human Services.

22. Laboratory Biosafety Manual. 1993. World Health Organization (WHO).

23. Collins, C.H. Laboratory-Acquired Infections: History, Incidence, Causes and Prevention. 3rd ed. 1993

24. L.I.Sly. Postal Regulations: Packing and Shipping of Cultures; Quarantine.

Appendix 2

Organisms Recognized to Exchange DNA through Known Physiological Processes

Members of any one sub-list are recognized to exchange DNA through known physiological processes, unless otherwise specified. Researchers should keep in mind that this list is not exhaustivey and feel free to suggest to the National Biosafety Committee for consideration, other pairs of organisms which have been shown or observed to exhibit natural exchange of genetic material, and merit inclusion to this list.

Unless otherwise specified, work with organisms that naturally exchange genetic material is considered 'exempt' under Category 1, if the donor and the recipient species are members of any one sub-list, and provided that the vector(s) do not incorporate DNA from organisms outside that particular sub-list.

sub-list A

Alcaligenes (2)
Campylobacter jejuni (2)
Campylobacter coli (2)
Campylobacter fetus (2)
Citrobacter (including Levinea)
Enterobacter
Erwinia
Escherichia
Klebsiella
Pseudomonas aeruginosa
Pseudomonas fluorescens
Pseudomonas mendocina (3)
Pseudomonas putida
Rhizobium (2)
Salmonella (including Arizona)
Shigella
Serratia marcescens
Yersinia enterocolitica

sub-list B

Bacillus amyloliquefaciens
Bacillus aterrimus
Bacillus globigii
Bacillus licheniformis
Bacillus nato
Bacillus niger
Bacillus pumilus
Bacillus subtilis

sub-list C

the following:
Streptomyces aureofaciens
Streptomyces coelicolor
Streptomyces rimosus
into Streptomyces sanguis

sub-list D

Streptomyces cyaneus
Streptomyces griseuss
Streptomyces venezuelae

sub-list E

one-way transfer of:
Streptococcus mutans or
Streptococcus lactis DNA
into Streptococcus sanguis

sub-list F

Streptococcus faecalis
Streptococcus mutans
Streptococcus pneumoniae
Streptococcus pyogenes

Streptococcus sanguis

sub-list G (2)

Bacillus cereus
Bacillus thuringiensis

Appendix 3

NBC Authorized Host/Vector Systems

The National Biosafety Committee regards and evaluates host/vector systems primarily on the basis of the potential for the aggregate to survive and to multiply in the open environment or generally, the viability of the system in conditions that may be encountered beyond that of the source laboratory. These biological containment and general biosafety concerns also take into consideration the natural tendency for the vector(s) to be transferred to non-target hosts, whether the conditions be, among others, the laboratory setting, field test plots or the immediate surroundings. An index of currently approved host/vector systems, patterned after the 1993 Australian GMAC list, follows.

Host/Vector Systems approved by the National Biosafety Committee on the basis of biological containment provided:

Class	Host	Vector
Bacteria	*Escherichia coli* K12, or a derivative thereof, which does not contain conjugative plasmids or generalized transducing phages	1. Non-conjugative plasmids 2. Bacteriophage • lambda • lambdoid • F1 (e.g. M13)
	Bacilhis subtilis or *Bacillus licheniformis*; Asporogenic strains with a reversion frequency of less than 10^{-7}	Indigenous *Bacillus* plasmids and phages with host ranges not inclusive of *B. cereus* or *B. anthracis*
	Pseudomonas putida strain KT 2440	Certified plasmids: pKT 262, pKT 263 and pKT 264
	Certified *Streptomyces* species: *S. coelicolor* *S. lividans* *S. parvulus* *S. griseus*	1. Certified plasmids: SCP2, SLP1, SLP2, PIJ101 and derivatives thereof 2. Acthinophage phi C31 and derivatives thereof
Class	Host	Vector

Fungi	Specified strains of *Neurospora crassa* modified to prevent aerial dispersion	No restriction
	Saccharomyces cerevisiae	No restriction
Tissue Culture	Mammalian, including human, cells	Non-viral or defective viral vectors (including retrovirus or retroviral/ helper combinations) that cannot infect mammalian cells
	Plant cell culture	Disarmed non-tumorigenic Ti plasmid vectors in *Agrobacterium tumefasciens* and non-pathogenic viral vectors

Source: Guidelines for Small Scale Genetic Manipulation Work, January 1993, GMAC, Department of Administrative Services, Australia

Researchers should be aware that endorsement of host/vector systems is only conditional and that the NBC reserves the right to withdraw, if having new, pertinent information or developments, its authorization of any host/vector system in practice.

Special Provision:

Situations in which the donor DNA is introduced into the host through electrical, mechanical or any other means without the use of biological vectors, shall be regarded and treated, by the IBC and the NBC, as approved host/vector systems provided that all of the following conditions are realized:

- The host represents any of the above approved host organisms or tissue cultures.
- The donor DNA is not derived from microorganisms which are root causes of diseases in humans, plants or animals.
- The donor DNA represents or comprises no more than 2/3 of any complete viral genome and is employed in such a manner as to disallow the possible regeneration of live viruses (as opposed to such work wherein the hosts carry the missing segments of viral genomes or whereby regeneration is made possible under the context of ensuing propagation sequences).
- The donor DNA does not code for proteins which regulate the growth of mammalian cells (e.g. product of oncogenes), for cytotoxic proteins, or for toxins, to vertebrates, with an LD50 of less than 100 *µg/kg*.

Such systems may be considered for an exempt status under Category 1 work.

Appendix 4

High-Risk, Virulent Toxins

Laboratory genetic manipulation work which entails the clon ing of gene sequences (or the breeding and propagation of microorganisms carrying such sequences) coding for toxins, to vertebrates, with an LD50 of less than 100 *µg/kg* must be authorized by the National Biosafety Committee before any work is allowed under way. A list of some such lethal toxins follows.

- Abrin
- *Bacillus anthracis* lethal factor
- *Bordetella pertussis* toxin
- Choilera - see *Vibrio choilerae*
- *Clostridium perfringens* epsilon toxin
- *Clostridium tetani* toxin
- *Corynebacterium diptheriae* toxins
- *Escherichia coli* heat labile (LT) enterotoxin and LT-like tox in
- Oxygen-labile haemolysins such as streptolysin O
- *Pasteurella pestis* murine toxins
- *Pseudomonas aeruginosa* exotoxin A
- Ricin
- *Shigella dysenteriae* toxin
- *Staphylococcus aureus* determinants A, B and F, alpha and beta toxin, and exfoliative toxin
- *Vibrio choilerae* (comma) 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

Source: NIH Federal Register Vol. 51, No. 88, May 1986 (Ap pen dix F) provided by the NIH Office of Recombinant DNA Activities

The NBC would appreciate any notices from researchers of other virulent toxins determined to have an LD50 of less than 100 *µg/kg*. The NBC requests supporting data on such toxins in order to confirm and accredit the findings.

Appendix 5

Framework of the Various Notification Forms

Project proposal and supplementary information forms may be obtained from the NBC Secretariat at the following address:

National Biosafety Committee
National Center for Genetic Engineering and Biotechnology (BIOTEC)
The 5th Floor, National Science and Technology Development Agency Building
Rama VI Road, Rajthevee
Bangkok 10400 THAILAND
Telephone: (066)-2-6448150-4
Facsimile: (066)-2-6448107-8

Either the *Project Proposal Form for Assessment of Laboratory Genetic Manipulation Work* or the *Project Proposal Form for Request of Exempt Status* (along with all attachments and supplements) will serve as the principal source of reference for the IBC and the NBC in the initial consideration and approval of research work regulated under these Guidelines. On the basis of information provided in, and of risks/concerns that may be inferred from these proposals, the IBC shall classify research work and determine additional biosafety measures to be adopted/implemented as necessary, including facility up grades and procedural amendments. Proposals intended for Category 3 work shall also be reviewed by the NBC, and whatever details pro vid ed will constitute the framework for NBC assessment and recommendations. Recognizing that IBC and NBC activities, in these initial stages of genetic manipulation practice, depend on the written forms submitted, researchers should be thorough yet concise, and clear as to their intentions, so that the committees may readily and fully understand the nature of proposed work. All important details should be included and as many additional sheets/pages may be attached as nec es sary to accommodate. Notable and exceptional intents should be stressed, ideally in the title or under the objectives. Particular care must be observed regarding phrasing-approval will be restricted to the *specific* experimental procedures and biological system com po nents identified so descriptions should be broad enough (though *never vague*) for the purposes of the research.

Project Proposal Form for Request of Exempt Status

1. Name and Institutional Address of Project Supervisor
2. Names of other Supervisors, Co-Investigators or Program/Section Leaders

    - Indicate Institutional addresses where different from (1).

3. Affiliations

    - Indicate names and addresses of the supporting institution, co-operating institutions and supervising Institutional Biosafety Committee.

4. Project Title
5. Project Objectives
6. Methodology and Protocol

    - Provide thorough yet concise descriptions of the main ex per i men tal procedures.

- Include timetable of activities.

7. Reasons why Project Merits Exempt Status

- Account with reference to standard Category 1 work under section 2.1.3 of the NCGEB Biosafety *Guidelines in Genetic Engineering and Biotechnology for Laboratory Work*, 1993.

8. Signature (of Project Supervisor) and Date

Project Proposal Form for Assessment of Laboratory Genetic Manipulation Work

Section A - Authorities and Outlook

1. Name and Institutional Address of Project Supervisor submitting proposal
2. Names of other Supervisors, Co-Investigators or Program/Section Leaders

- Indicate institutional addresses where different from (1).

3. Affiliations

- Indicate names and addresses of the supporting institution, co-operating institutions and supervising Institutional Biosafety Committee.

4. Project Title
5. Project Objectives

Section B - Materials and Methods

6. Methodology and Protocol

- Provide thorough yet concise descriptions of the main experimental procedures.
- Include a timetable of activities.
- Describe any special precautions/safeguards to be adopted.

7. Details of the Biological System

7.1 Account of the Origin (biological source) of donor DNA

7.2 Characteristics of the donor DNA

7.3 Description of the Host Organisms or Tissues

7.4 Description of the Vectors or Methods for transfer of donor DNA into the Host

7.5 Characteristics of the Host/Vector System(s), as applicable (for each, describe)

- Predicted Stability of Introduced Genetic Traits.
- Identification Characteristics or Markers.
- Hazard and Biological Containment.
  [ ] NBC Authorized [ ] Not Authorized

7.6 Ecological Context (Auto-Ecology) (for each host/vector system, assess)

- Viability in Open Environments.
- Natural Crossing Possibilities to Related Species.

8. History of Prior Work with Components of the Biological System
9. Intended Classification
[ ] Category 2 [ ] Category 3
10. Laboratories and Facilities where Work will be Conducted (for each, indicate)

- Complete Address.
- Work to be Conducted.
- Certified Physical Containment (Biosafety) Level.
  [ ] C1 [ ] C2 [ ] C3 [ ] C1A [ ] C2A [ ] PH1
  [ ] PH2 [ ] PH3
  [ ] Other (please specify)
- Special Containment/Safety Features.
- Approval (Contract) for Use.

11. Intended Date of Commencement; Expected Date of Completion

Section C - Personnel Involved with Research Work Pro posed

12. Details of Personnel

- Name, Qualifications and Experience.
- Responsibilities and Duties.
- Medical History.

13. Signature (of Project Supervisor) and Date

Instructions for Completion of the *Project Proposal Form for Assessment of Laboratory Genetic Manipulation Work*

The project supervisor must submit two typed, completed project proposal forms to the supervising IBC (one of which shall be forwarded to the NBC for information) and should retain a copy for records and reference. For work supported by two or more institutions, all IBCs of authority must be notified.

Project proposal forms must be signed and dated by the project supervisor to be received by the IBC and the NBC. For research work employing multiple project supervisors or head researchers, the name and professional address of the supervisor preparing and submitting the proposal should be indicated under heading (1). Said individual shall sign and date both proposals before submission to the IBC of authority.

As many additional sheets/pages may be attached as necessary. Incomplete proposals will delay IBC endorsement as further information is sought.

Names and Addresses

Names, institutional addresses and addresses of institutions, laboratories, containment facilities and biosafety committees should be given in full. Ideally, telephone numbers, facsimile, e-mail accounts etc. should be provided along with postal addresses. Foreign establishments must indicate both contacts in Thailand and abroad.

Intentions to relocate must be promptly referred to the IBC so contact/links can be assured at all times.

Project Title and Objectives

Notable and exceptional intents should be indicated in the title or under the objectives.

Short and long term objectives should be state separately if the research work proposed is likely to continue for several years. Distinguishing immediate from remote aims, together with providing a timetable of activities (under the Methodology and Protocol section), will permit the IBC to assess the proposal in sequence. As such, where the entire proposal does not merit endorsement (or where endorsement of later stages depends on the results of early work), the IBC may approve particular initial stages rather than having to reject the proposal as a whole--allowing preliminary work to begin as the proposal is revised (or as results are compiled). Researchers should clearly indicate which parameters of the work requires primary endorsement.

Ideally, the objectives should communicate, to some considerable extent, a service to the welfare of the community or the environment, local or global. Justify hazardous or high-risk work by relating why the ends cannot be attained through conventional or alternative practices that offer less risk.

Methodology and Protocol

Only a concise--but thorough--description of the main experimental procedures is required. A timetable of activities should be included to allow the IBC liberty in assessing the proposal in se quence. Detail any special precautions/safeguards to be adopted, with references to the specific risks and concerns identified in initial risk assessments.

Materials

For work with multiple donor DNA, hosts and/or vectors, indicate when and how each shall be used. As with providing a time table of activities and distinguishing short and long term aims, clarify ing these points will allow the IBC to assess the project in stages or modules.

Some details of the relevant history of prior work with components of the biological system should be provided under heading (8), including track records of safety and biological containment. In di cate whether the DNA, hosts and vectors concerned are commonly or rarely subjected in regulated work. Above all, identify any problem DNA, host or vector with a history of unsafe use (e.g. often realizing the hazards determined in initial risk assessments). If related host/vector systems have been field tested or released, a summary of the results/analysis would be appropriate.

Donor DNA

The origin of all donor DNA should be specified--scientific name and strain of the biological source(s), whether procured or construct ed by the research team, and if procured, who made it. Researchers must account for how donor DNA was or will be constructed/cloned and should make clear as to whether several genes or species are in volved. Some details of the biological source(s) are appropriate, for instance, whether a local or exotic strain and patterns of local distribution, particularly if imported.

All important characteristics of donor DNA should be listed. Uncharacterized donor DNA should be so indicated otherwise, the IBC shall expect a review of the known functions of target genes through to the known functions of the proteins encoded.

Host Organisms or Tissues

The scientific name and strain of all host organisms and biological sources of host tissues need to be specified. In addition, regarding host tissues, researchers should briefly account for how the tissue cultures were or will be prepared/grown. For work using as hosts, genetically modified or constructed organisms, also include a review of the genetic manipulation work involved.

List all substantial hazards conveyed by host organisms or tissues, particularly, regarding pathogenicity and infectivity. Other details of the hosts may be appropriate, for instance, geographic dis tri bu tion and biologically active compounds secreted.

Vectors

Work with biological vectors requires a concise description of the known vector properties (e.g. host range) in addition to nomenclature or identification. As with host organisms/tissues, list all sub stan tial hazards borne, particularly, regarding pathogenicity and infectivity. For vectors which are genetically modified or constructed (e.g. retroviral vectors), provide some details of the construct and methodology involved. A genetic map would also be appropriate.

In the case of electroporation, and other electrical or mechanical methods for transfer of donor DNA into hosts, only a brief statement is expected.

Host/Vector System(s)

Researchers should elaborate, somewhat, on the predicted stability of introduced genetic traits (including, the localization and copy number of target genes, introduced gene expression, frequency of re ver sion to wild type characteristics) and the form of heredity of the target phenotype(s). Where applicable, also assess the likely stability of plasmids, phages, viruses, etc. in host organisms/tissues.

Identification characteristics or markers should be detailed for IBC and NBC references.

A concise--but thorough--risk assessment for the host/vector system(s) proposed is required. Specify whether the level of biological containment provided classifies each system as 'NBC Authorized' or 'Not Authorized' (see Appendix 3 of the NBC Biosafety Guidelines in Genetic Engineering and Biotechnology for Laboratory Work).

Auto-Ecology

Under 'Ecological Context', substantiate the level of biological containment provided by each host/vector system involved. Researchers need to briefly assess the viability of host/vector systems in the open environment (particularly, the natural tendency for invading wild populations, and for developing into pests or weeds) and indicate any factors (including genetic modifications) which might limit growth, reproduction and survival. Additionally, the IBC shall expect a review of the natural crossing possibilities to, or possibilities for ex change of genetic material with related species/natural variants.

Any details of the evolutionary potential should be presented under this heading.

Laboratories and Facilities

Clarify which phases of the work will be conducted in each of the laboratories and facilities identified, specify the certified con tain ment level and describe any special containment/safety features offered. Researchers must indicate whether permission has already been obtained for use, and if so, indicate the period of time awarded and attach written confirmation.

Details of Personnel

Attach a CV for each personnel involved in the proposed work, covering personal qualifications (e.g. education, training, professional history) and relevant research experience. Ideally, the general responsibilities of each individual should also be noted, so that the IBC may assess, on a case-by-case basis, whether personnel are adequately prepared and capable to handle the duties assigned.

It is essential that brief medical histories of all personnel at risk be included.

Commercial-in-Confidence

Researchers who wish to restrict access to information of commercial significance (e.g. trade secrets or confidencial business reports) provided to the IBC and NBC in projects poroposals, should mark relevant material or portions "Commercial-in-Confidence".

Supplementary Information Form for Laboratory Genetic Manipulation Work on Whole Plants

Attach a copy to both project proposals to be submitted for IBC notification.

Attach as many additional sheets/pages as necessary.

Section A - Supplementary Information

1. Name and Institutional Address of Project Supervisor submitting proposal

2. Affiliations

- Indicate names and addresses of the supporting institution, co-operating institutions and supervising Institutional Biosafety Committee.

3. Project Title

4. Further Details of the Biological System

4.1 Are the experimental plants noxious weeds?
[ ] Yes [ ] No
If yes, elaborate on the ecological context:

- Reproductive Cycle and Evolutionary Potential.
- Dispersal, Proliferation and Persistence in Open Environments.
- Factors which might limit growth, reproduction and survival .
- Natural Crossing Possibilities (to wild populations).
- Noxious Characteristics.

4.2 Are the experimental plants closely related to, or conspecific with wild material which are noxious weeds?
[ ] Yes [ ] No
If yes, indicate which species/strains/natural variants; and elaborate on the ecological context of each:

- Reproductive Cycle and Evolutionary Potential.
- Dispersal, Proliferation and Persistence in Natural Habitats.
- Factors which might limit growth, reproduction and survival.
- Natural Crossing Possibilities (particularly to experimental plants).
- Noxious Characteristics.

4.3 Are the micro-organisms involved harmful to humans, plants or animals?
[ ] Yes [ ] No
If yes, elaborate on the harmful agent (e.g. pathogenic or infectious determinant? toxic substance?) and the known or likely modes of transmission. Indicate any potential to cause epidemics.

5. Further Details of the Methodology

5.1 Substrate for use in cultivation? [ ] Soil
[ ] Soil Substitute (specify)
Describe the sterilization procedures.
(Complete only if genetically manipulated plants are to be grown)

5.2 Plans for Cultivation of Genetically Manipulated Plants

- Developmental Stage(s) Targeted and Intentions to Breed .
- Arrangements for containment of plants and plant materials (spores, seeds, pollen, vegetative materials).
- Arrangements for disposal of plants and plant materials; wastes and by-products which may contain viable plant materials.

6. Provide details of equipment and facilities for use in cultivation.

7. Additional information relevant to the assessment of this work.

8. Signature (of Project Supervisor) and Date

Section B - IBC Assessment

9. Assess, on a separate sheet(s) of paper, the supplementary information provided for the proposed work, and attach to the completed IBC Form for Assessment of a Proposal to carry out Laboratory Genetic Manipulation Work

10. Signature (of IBC Chairperson) and Date

Supplementary Information Form for the Engineering of Transgenic Animals

(Considerations in the Design of Transgenic Animal Houses and in the Choice of Animal Containment Levels[*])

Attach a copy to both project proposals to be submitted for IBC notification

Attach as many additional sheets/pages as necessary.

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[*] Animal containment levels: CIA, C2A, equivalent or higher

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Section A - Supplementary Information

1. Name and Institutional Address of Project Supervisor submitting proposal

2. Affiliations

- Indicate names and addresses of the supporting institution, co-operating institutions and supervising Institutional Biosafety Committee.

3. Project Title

4. Further Details of the Experimental Animals

4.1 Nomenclature

- Class, scientific name and strain.
- Indicate whether a local or an exotic species.

4.2 Number of Experimental Animals
(schedule of the approx. no. of animals to be handled each session, or at any one time)

4.3 Developmental Stages Targeted
(stages in life cycle involved, for example, "embryo only" or "through reproductive age")

5. Further Details of the Genetic Manipulation

5.1 Does genetic manipulation involve
[ ] germline or [ ] somatic cells?

5.2 Characteristics of donor DNA

- Origin and construct (with references to biological source(s) and to proposal(s) covering preparation of the DNA).
- Characterization and history of prior use in genetic manipulation (with references to published work).
- Physiological traits intended to be conferred (e. g. production of alien, biologically active compounds, heightened resistance, altered appearance and productivity).

5.3 Method for Introduction of donor DNA
[ ] electrical (specify) [ ] mechanical (specify)
[ ] biological vector if a biological vector is involved, describe:

- Construct and characterization (with references to published work and to proposal(s) covering preparation of the vector).
- History of prior use in genetic manipulation and recommended containment level.

5.4 Heredity of Introduced Traits

- Reproductive capacity of experimental animals
- Assessed heritability of transgene(s).
- Expected form of heredity of target phenotype(s).
  (Complete this section only if other animals will be housed in the same facilities, but for other work)

6. Concurrent Use of Transgenic Animal Houses

6.1 Number and Composition of the Other Animals (number of animals of different species/strains)

6.2 Basic Nature of the Other Work (use of the other animals, for example, in infectious disease work or in separate genetic manipulation work)

6.3 Arrangements to keep transgenic animals separate from the other experimental animals (physical and temporal provisions)

6.4 Arrangements to identify and to account for individual animals

7. End Use, Care and Disposal

7.1 Intentions to breed, if animals will be reared to full maturity (plans/scheme for projected crosses)

7.2 End Use/Application of transgenic animals (only a brief description is necessary)

7.3 Arrangements for the care or disposal of transgenic animals at the conclusion of work

8. Additional information relevant to the assessment of this work.

9. Signature (of Project Supervisor) and Date

Section B - IBC Assessment

10. Assess, on a separate sheet(s) of paper, the supplementary information provided for the proposed work, and attach to the completed *IBC Form for Assessment of a Proposal to carry out Laboratory Genetic Manipulation Work*.

11. Signature (of IBC Chairperson) and Date

Appendix 6

Framework of the IBC Assessment Form

The IBC Form for Assessment of a Proposal to carry out Laboratory Genetic Manipulation Work serves, above all, to guide the Institutional Biosafety Committees in the consideration and evaluation of project proposals. These forms are meant to provide a framework for IBCs in assessing the experimental parameters of proposed research-leading up to the decision on whether to endorse the work at hand and culminating in the preparation of amendments and provisions to be adopted as necessary. The IBCs must be clear in their evaluation of each component of the experimental system identified in the assessment form. Additionally, the committees should be thoughtful and thorough in drafting the various amendments and provisions to ensure an acceptable standard of biosafety for laboratory work under consideration. Regarding proposals for Category 3 work, special attention should be paid to determine which issues require NBC advice. Completed IBC assessments shall be submitted to the NBC, together with corresponding project proposals, and the efforts of the committee will in turn, guide the NBC in reviewing the work proposed, as required.

Institutional Biosafety Committee

Form for Assessment of a Proposal to carry out Laboratory Genetic Manipulation Work

Section A - IBC Assessment of Project Proposal

1. Name and Institutional Address of Project Supervisor who submitted the proposal

2. Affiliations

- Indicate names and addresses of the supporting institution, co-operating institutions and supervising Institutional Biosafety Com mit tee.

3. Project Title

4. Experimental Parameters

- l Indicate whether approved, not approved or inconclusive (in suf fi cient information provided); and
- l Include a concise explanation for IBCs position on each of the following.

4.1 Project Objective and Methodology

4.2 Biological System

4.3 Physical Containment (Biosafety) Facilities

4.4 Details of Personnel

- Experience and Expertise
- Training and Instruction
- Health
- Other (please specify)

5. Classification of Proposed Work

- Intended (as indicated by the Project Supervisor on the Project Proposal Form for Laboratory Genetic Manipulation Work).
  [ ] Category 2 [ ] Category 3
- As Assessed by the IBC
  [ ] Category 2 [ ] Category 3

6. Physical Containment (Biosafety) Level
(for each laboratory and facility where work will be conducted, indicate)

- At Hand (as certified by the IBC or NBC).
  [ ] C1 [ ] C2 [ ] C3 [ ] C1A [ ] C2A [ ] PH1
  [ ] PH2 [ ] PH3 [ ] Other (please specify)
- Required by the IBC (for the purposes of the work to be conducted).
  [ ] C1 [ ] C2 [ ] C3 [ ] C1A [ ] C2A [ ] PH1
  [ ] PH2 [ ] PH3 [ ] Other (please specify)

7. The project proposal form attached has been reviewed by the IBC and as assessed above, the committee [ ] *endorses* / [ ] *does not endorse* the research work proposed.

Section B - IBC Recommendations

(Complete this segment only if Project Proposal does not receive IBC endorsement)

8. The following conditions and amendments must be adopted for the research work proposed to receive future endorsement from the IBC.

(Complete this segment only if Project Proposal receives IBC endorsement)

9. The following special provisions must be adopted and implemented in conjunction with the NCGEB Biosafety Guidelines in Ge net ic Engineering and Biotechnology for Laboratory Work, 1993 dur ing the conduct of research work.

Section C - IBC Requests to the National Biosafety Committee

(Complete this section only if Project Proposal is intended for Category 3 work)

10. The project proposal form attached has been reviewed by the IBC and as assessed above, the committee requires and requests spe cif ic NBC advice regarding the following.

11. Signature (of IBC Chairperson) and Date

Instructions for Completion of the *IBC Form for Assessment of a Proposal to*

carry out Laboratory Genetic Manipulation Work

The IBC must submit a typed, completed assessment form to the NBC, attached to the corresponding project proposal, and should retain a copy for records and reference. Assessment forms must be signed and dated by the IBC Chairperson.

A clear and concise explanation is required for the IBCs position on each of the experimental parameters identified in the as sess ment form. The NBC shall expect some justification on IBC decisions to approve or not to approve the various components of the experimental system proposed. Where inconclusive, the IBC must indicate what information is lacking. As appropriate, references should be made to the relevant sections of the NBC *Biosafety Guidelines in Genetic Engineering and Biotechnology for Laboratory Work.*

Details of personnel need to be checked by the IBC but the relevant attachments should not be forwarded to the NBC.

Some Specific Provisions

Proposals for work awarded exempt status under Category 1

IBC assessments and corresponding project proposals need not be forwarded to the NBC for information.

Proposals for work assessed as Category 2

The IBCs may authorize or commission research work immediately, upon endorsement of the project proposals, but must in form the proponents of any additional conditions to be adopted or guide lines to be observed, beforehand. IBC assessments should be attached to the top sheet of the corresponding project proposals and submitted to the NBC soon afterwards.

Proposals for work assessed as Category 3

IBC assessments should be attached to the top sheet of the corresponding project proposals and submitted to the NBC at the ear li est possible. The IBCs may not authorize or commission research work (even if the committees initially endorse the proposals) until complete reassessment of project proposals following receipt of NBC ad vice and recommendations.

Appendix 7

Requirements for Containment Level C1

A Note on Physical Containment

Containment Levels C1, C2 and C3 are the different classes or grades of physical containment which may be afforded by genetic manipulation laboratories/facilities. The fundamental objective of physical containment* is to prevent undue exposure of the laboratory worker, the community or the environment to regulated material and as such, is achieved primarily through the adoption of proper lab o ra to ry procedures and containment equipment. Special laboratory de sign provides a secondary means of protection and figures prom i nent ly in the containment of the more hazardous, genetically manipulated agents.

Three standard combinations of laboratory procedures, containment equipment and laboratory design give rise to the three basic levels of physical containment, detailed hereinafter. Where al ter na tive combinations or supplementary precautions may better address the concerns of research work at hand, the project supervisors and the IBCs involved shall be primarily responsible for the preparation of specific and varied provisions, as is appropriate (e.g. immunization requirements, microwaving of infectious wastes, provisions for a cen tral vacuum system).

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* Biological containment is a rather different matter (Appendix 3) but may often supplement physical containment.

Laboratory Procedures

1. When work is in progress, laboratory doors are closed and entrance is restricted at the discretion of the project supervisor.

2. Personnel wear, over street clothes, laboratory coats or gowns which are removed before leaving the laboratory.

3. Eating, drinking, smoking and applying cosmetics are all prohibited.

4. Food and drinks may not be stored in the work area at any time.

5. Mouth pipetting is prohibited; mechanical pipetting instruments are used instead.

6. All procedures are performed cautiously to minimize the cre ation of aerosols. Use of sonication or vortex machines, and other procedures which tend to generate aerosols, should be done in biological safety cabinets.

7. Personnel wash their hands with liquid soap and warm water after handling experimental organisms and cultures, and before leaving the laboratory.

8. Instruments used in culture work or with contaminated ma te ri al are to be disinfected after use, if the instruments are not readily steam sterilized. For glassware, a hypochlorite solution made up with 5000 ppm of chlorine (dilute household bleach 1:8 and allow to react for at least 30 minutes), prepared daily, provides a suitable disinfectant.

9. Floors, work benches and surfaces are decontaminated with a suitable disinfectant after each session and immediately after any spill of viable material. Other than the basic hypochlorite solution, disinfectants must be prepared and used according to the manufacturer's instructions.

10. All microbiological wastes are decontaminated (preferably, autoclaved) before disposal.

11. Regulated materials are packaged within securely- sealed dou ble containment units (section 5.1) before being removed from the laboratory to autoclaves or rooms and facilities elsewhere. These units must be opened to allow for thorough penetration of steam during autoclaving.

12. An appropriate pest control program is in effect, as su per vised by a licensed pest

control operator.

Please Note: All work to be performed in a C1-level laboratory must observe C1-level procedures regardless of whether the work in volves genetic manipulation.

Containment Equipment

1. Biological Safety Cabinets are provided (particularly, where laboratory operations tend to generate considerable amounts of aerosol)

As terms of reference, specifications for and use of biological safety cabinets shall comply with the following Australian Standards:

- A.S. 2252.1, *Biological Safety Cabinets (Class I) for Personnel Protection*
- A.S. 2252.2, *Laminar Flow Biological Safety Cabinets (Class II) for Personnel and Product Protection*
- A.S. 2647, *Biological Safety Cabinets - Installation and Use* (which also addresses the problem of frontal airflow disturbances and details protocols for decontamination)

2. There is close access to a steam sterilizer.

Laboratory Design

1. A closet, for laboratory coats and gowns, is provided next to the exit.

2. Room and work surfaces are smooth, impervious and re sis tant to attack by standard acids, alkalis, organic solvents and moderate heat.

3. The laboratory can be easily cleaned and thoroughly decontaminated; gaps and spaces between room surfaces, benches, furniture and equipment are accessible for wipe down. Ideally, benches, furniture and equipment should be anchored and sealed to room surfaces. False ceilings should be avoided.

4. Laboratory windows that open are fitted with fly-screens.

5. The laboratory entrance is labeled with an official sign designating the certified level of containment (available from the IBC, pending certification), with the universal biohazard symbol and, when work is in progress, with a notice detailing the entry requirements and pro ce dures. Names and contacts (e.g. postal address, telephone and pager number) of the responsible authorities should be clearly indicated.

6. Signs are posted within the laboratory, outlining the ap pro pri ate operating procedures, contingency plans and instructions for upkeep and maintenance.

7. Freezers, refrigerators, liquid nitrogen tanks and other appliances for the storage of recombinant DNA or of manipulated genetic material, are labeled with the universal biohazard symbol.

Appendix 8

Requirements for Containment Level C2

Containment level C2 provides a moderate level of physical containment through an

even blend of proper laboratory procedures, suitable containment equipment and special laboratory design.

Laboratory Procedures
(all of containment level C1, and the following...)

1. Persons enter the laboratory for cleaning, audits, repairs and other activities at the discretion of the project supervisor (or the bi o log i cal safety officer) and only after laboratory surfaces have been properly disinfected.

2. Laboratory coats, gowns and protective clothing are placed in sealed bags or boxes (which may be readily steam sterilized), and brought to an autoclave for decontamination after each session and before laundering. Laboratory clothing shall not be worn outside the laboratory.

3. Sonication, Vortex and other machines/instruments that generate aerosols are kept and used only in biological safety cabinets.

4. Work surfaces and biological safety cabinets are decontaminated with formaldehyde gas after each session and after major spills of viable material.

5. Laboratories are inspected and serviced periodically. Screens, filters, ventilation and drainage systems are cleaned regularly.

Please Note: No other work must be done simultaneously with work requiring C2 levels of physical containment.

Containment Equipment
(all of containment level C1, and the following...)

1. Independent room exhaust fans are installed to achieve room pressure control. Exhaust fans must be equipped with a variable-speed drive and should be able to maintain a minimum air pressure dif fer en tial of 50 pascals. Discharge should be through a high-efficiency ex haust filter...

2. Exhaust filters are HEPA class, outfitted with a metal separator and rigged to a supplementary prefilter, of the same specifications as replacement air filters (#3). Exhaust filters will have a modular, metal framework and will not make use of fluid or grease seals. As the norm, unit specifications must comply with Australian Standards 1324 clause 4.3.1 (b) and performance audits must observe A.S. 1807.6 test ing guidelines.

3. Channels to draw in replacement air are engineered with contract ible apertures and high-efficiency filters that prevent back-flow. As the norm, filter specifications must comply with Australian Stan dards 1324 for Type 1, Class A or Class B models, and have at least a 90% arrestant efficiency against Test Dust No. 2 under A.S. 1132.5 testing guidelines.

4. An airlock is provided at each access, to maintain a reduced laboratory air pressure during entry and exit. The basic design in cor po rates a pair of outward-opening doors, arranged in sequence so that a small chamber rests in-between. Each door must be self-closing and fitted with a viewing panel. The outer door requires a security lock.

5. Manometers are installed to monitor air pressure drop across exhaust prefilters and a Magnehelic type differential pressure gauge is outfitted to measure room negative

pressure. Ideally, climate-control switches, exhaust fan speed dials and replacement air aperture-adjustment controls should be affixed next to the gauge to support manual room pressure control.

6. Special protective clothing, head covers, overshoes, gloves, molded surgical masks and respirators are provided as required.

Laboratory Design (all of containment level C1, and the following...)

1. The laboratory is isolated from and does not open onto public walkways.

2. Laboratory windows are closed and sealed; walls, ceilings and floors are substantially airtight.

3. Access to roof spaces above the laboratory, and to other enclosing or contiguous voids, is restricted so as not to unwittingly com pro mise structural integrity.

4. Room and work surfaces are resistant to attack by disinfectants, gases and other agents used in the laboratory.

5. Ventilation designs allow the laboratory to operate at a requisite room pressure of 50 pascals below external air pressure, when all doors are closed; during entry and exit through airlocks, internal air pressure shall remain at least 25 pascals below external pressure.

6. Fans, filters and ventilation shafts are positioned to facilitate inspection and performance audits.

7. Architectural and structural requirements accommodate the need for laboratory pressure to be maintained below external air pressure. An airlock is provided at each access. Laboratory surfaces and windows can withstand the variable air pressure loads imposed by ventilation fans during all modes of operation.

8. The various sensing devices set off or sound an alarm to indicate loss of room pressure control.

9. A fan coil cooling system, using chilled water or a refrigerant as the cooling medium, is in place, where exhaust ventilation rates alone cannot sufficiently offset room heat loads. Care must be observed in setting up the system to avoid airflow disturbances in front of biological safety cabinets.

10. The laboratory can be sealed-off to allow for decontamination of the entire room with formaldehyde gas. Ventilation shafts, exhaust ducts and replacement air apertures can be closed-off (e.g. by way of dampers and cover plates). Ideally, provisions for remote power switches should be made to allow for the safe generation of fumes.

11. Systems are in place to treat formaldehyde gas, generated for decontamination of work surfaces and biological safety cabinets, to allow for safe discharge into atmosphere.

Appendix 9

Requirements for Containment Level C3

Containment level C3 offers a higher level of physical containment and necessitates

much more rigorous conditions than does containment level C2. A medley of additional engineering and architectural requirements is warranted—ranging from the installation of various and duplicate items of machinery inside the work area, to the special design of laboratory ventilation and drainage systems, to be separate from those servicing the rest of the complex. Heightened laboratory performance would likely be extended to include directional airflow and emergency life-support. Further provisions for personnel safety might involve advanced protective clothing (e.g. one-piece positive pressure suits) and chemical showers. Additional operating procedures must be adopted as well, complementing the equipage and construct of C3-level facilities.

Institutions, with plans to support C3-level laboratories, must confer with the NBC to determine and fulfill the relevant and extensive requirements, as dictated by the nature of existing risks and concerns. No discrete criteria are given.

Appendix 10

Requirements for Biosafety Level PH1 in Plant Glasshouses

Containment in Plant Glasshouses

Biosafety levels PH1, PH2 and PH3 are the different classes or grades of physical containment which may be afforded by plant glasshouses and greenhouses. Although emphasis is on the containment of experimental plants and plant materials, the extent of concern encompasses the need for containment of other regulated material involved as well (e.g. donor DNA, biological vectors, tissue cultures). As with physical containment in laboratories, containment in plant glasshouses is achieved through the adoption of proper operating procedures, containment equipment and room design.

Biosafety level PH1 provides the lower level of containment but for the most part, conditions are suitable for Category 2 genetic manipulation work on whole plants. PH1-level plant glasshouses may likewise accommodate Category 3 work with whole plants, but often require supplementary operational routines and special room design (including minor structural modifications) for such purposes.

Design and Equipment

1. Glasshouses have concrete floors.

2. Windows and other openings along walls and ceilings (e.g. vents) are fitted with fine screens (standard: thirty gauge 30/32 mesh wire gauze).

3. Drainage conduits are engineered to deter rodents, insects and other pests.

4. An antechamber is required at each access, except for those that lead directly to another containment facility. Antechambers must offer substantial pest deterrence features (e.g. automatic pesticide spray/aerosol devices, supplemented with conventional sticky pest strips). The innermost door should open inwards and be self-closing. A closet, for protective clothing and apparel, is provided within.

5. Biological safety cabinets are provided, as specified for containment level Cl. (Appendix 7)

6. The glasshouse can be easily cleaned and thoroughly decontaminated; gaps and spaces between room surfaces, benches, forniture and equipment are accessible for

wipe down.

7. The entrance to the glasshouse is labelled with an official sign designating the certified biosafety level (available from the IBC, pending certification), with the universal biohazard symbol and, when work is in progress, with a notice detailing the entry requirements and procedures. Names and contacts (e.g. postal address, telephone and pager number) of the responsible authorities should be clearly indicated.

8. Signs are posted within the glasshouse, outlining the appropriate operating procedures, contingency plans and instruction for upkeep and maintenance.

Operational Routines

1. When work is in progress, all doors to the glasshouse are closed and entrance is restricted at the discretion of the project supervisor. On the whole, entry is reserved for those whose presence is warranted in program or support activities. Entry requirements are binding and IBC authorisation must be obtained for access.

2. All doors to the glasshouse are kept locked during off-hours, to prevent inadvertent access.

3. Personnel wear, over street clothes, laboratory coats or gowns which are removed before leaving the laboratory.

4. Eating, drinking, smoking and applying cosmetics are all prohibited.

5. Food and drinks, for human consumption, may not be stored in the work area at any time.

6. All procedures are performed cautiously to minimize the creation of aerosols. Those procedures which tend to generate aerosols should be done in biological safety cabinets.

7. Personnel wash their hands with liquid soap and warm water after handling experimental organisms and cultures, and before leaving the glasshouse.

8. Instruments used in culture work or with contaminated material are to be disinfected after use, if the instruments are not readily steam sterilized. For glassware, a hypochlorite solution made up with 5000 ppm of chlorine (dilute household bleach l:8 and allow to react for at least 30 minutes), prepared daily, provides a suitable disinfectant.

9. Floors, work benches and surfaces are decontaminated with a suitable disinfectant after each session and immediately after any spill of viable material. Other than the basic hypochlorite solution, disinfectants must be prepared and used according to the manufacturer's instructions.

10. All biological wastes (e.g. plant materials, tissue cultures), soil and soil substitutes and the containers of viable or contaminated material are inactivated or sterilized before disposal.

11. Regulated materials are packaged within securely-sealed double containment units (section 5.1) before being removed from the glasshousese to rooms and facilities elsewhere. Viable plants and tissues shall only be delivered to a certified containment facility. Transfer to other institutions must be approved by the supervising IBC.

12. Experimental plants are treated as if they all incorporate foreign genetically-manipulated DNA, regardless of the true extent of genetic modification.

13. An appropriate pest control program is in effect, as supervised by a licensed pest control operator. Signs of arthropod infestation must be monitored scrupulously, with particular attention on mites, which are too small to be effectively screened out. Experimental plants, whilst permitting, should be treated regularly with a systemic insecticide. The plant house itself, should be subject to a fumigation regimen, tailored to the existing concerns.

14. Glasshouses are inspected and serviced periodically. Screens, filters, ventilation and drainage systems are cleaned regularly.

Please Note: Plant glasshouses/greenhouses designed, and intended to support genetic manipulation work, should not be used for purposes other than such.

Appendix 11

Requirements for Biosafety Level PH2 in Plant Glasshouses

Biosafety level PH2 provides a moderate to high level of plant containment, as appropriate to the needs and concerns of the work at hand. Although there are basic requisites, there is no real standard combination of room design, equipment and operational routines. This flexibility allows for a range of containment environments, specifically addressing the risks involved and the media by which these risks are borne. With this in mind, principal investigators and supervising IBCs should conduct thorough risk assessments before taking any further measures to accommodate plant research work requiring PH2-levels of containment.

The NBC certifies PH2-level plant glasshouses on a case-by-case basis, considering the nature of work to be supported by the facility. On occasion, upon reviewing project proposals for PH2-level glasshouse work, the NBC may decide that proposed PH2-levels of containment are excessive or extravagant, and recommend the alternative use of PHI level facilities with additional, imposed constraints.

An index of the basic requisites for biosafety level PH2, follows:

Design and Equipment
(all of biosafety level PHI, and the following...)

1. The joins between structural components are sealed. Inasmuch as possible, bench tops and work surfaces are seamless as well

2. Ideally, transparent panels are constructed of impact-resistant material (e.g. methyl-methacrylate, commercially licensed as *Perspex*) or reinforced glass. If ordinary window glass is to be used, a hail-stone screen or some other encasing is outfitted to shield the panels.

3. Air supply and exhaust ducts are fitted with fine screens (standard: thirty gauge 30/32 mesh wire gauze)

4. A washbasin or sink is provided in the antechamber or in the glasshouse, next to the entrance. Where a laboratory adjoins and leads directly to the glasshouse, the washbasin may be located in the laboratory, by the connecting passageway.

Operational Routines
(all of biosafety level PH1, and the following...)

1. Before or upon entering the glasshouse, personnel wash their hands with liquid soap and warm water in the washbasin, provided inside the antechamber or by the entrance.

2. In the antechamber, before entering the glasshouse, personnel don special protective clothing (e.g. gowns/boiler suits), and put on head covers, overshoes, gloves, molded surgical masks and respirators as necessary.

3. Protective clothing and apparel are removed upon leaving the work area and kept in a closet provided in the antechamber. They are decontaminated and laundered regularly, after each session.

4. Materials and equipment, to be brought into or out of plant glasshouses, are treated to kill arthropods and arthropod larvae, and to destroy eggs and any other active or dormant stages of the arthropod life-cycle. Treatment of soil is not always practical, so use of soil should be avoided. Soil substitute, which may be readily decontaminated, must always be treated.

5. Whenever possible, use soil substitute for cultivation. Use of soil is discouraged.

Appendix 12

Requirements for Biosafety Level PH3 in Plant Glasshouses

Biosafety level PH3 offers a most rigorous level of containment in plant glasshouse work. To maintain such stringent accommodations through the duration of work, a number of special engineering and architectural requirements is heavily warranted, as with laboratory containment level C3. Additional operating procedures and safe ty provisions should be adopted to complement the special design of glass house facilities and systems.

Along the same lines as biosafety level PH2, no discrete criteria are given for biosafety level PH3. Selection of room design, containment equipment and operational routines shall be consistent with the concerns exposed in initial risk assessments, but the following ba sic requisites must always be observed:

Design and Equipment
(all of biosafety level PH2, and the following...)

1. All transparent panels are constructed of impact resistant material or reinforced glass. Regular window glass is not used, even if provisions are made to outfit hail-stone screens or any other encasing.

2. Ventilation designs allow the glasshouse to operate at a requisite room pressure of 50 pascals below external air pressure. Manometers are installed to monitor air pressure differentials and a Magnehelic type differential pressure gauge is outfitted to measure room negative pressure. These various sensing devices set off or sound an alarm to indicate loss of room pressure control.

3. Air supply and exhaust ducts are fitted with HEPA filters.

4. Drainage water from the glasshouse and antechamber collect in a central tank. Plant

glasshouses and antechambers have im per vi ous floors and lower-walls, and watertight doors so that accidental spills and cleaning fluids may be made to drain into the central tank.

5. Antechambers are equipped with an autoclave.

6. Washbasins and sinks for hand washing may be operated by foot, by elbow or automatically. Disinfecting footbaths are provided before the internal antechamber doors, leading into the glasshouse.

7. Plant glasshouses and antechambers can be sealed-off to allow for space decontamination or fumigation of the entire facility against microorganisms and arthropods.

8. Mechanical units, such as generators and water pumps, are positioned outside the glasshouse to allow for maintenance and repair without entry into controlled areas. Ideally, those equipment which receive or process experimental materials or wastes from glasshouses, should be specially engineered to stifle leaks and preclude escape of viable material. Otherwise, such equipment must be contained under airtight constructions, which may be readily decontaminated. An egress shall be designed to provide access for servicing.

Operational Routines
(all of biosafety level PH2, and the following...)

1. All materials and equipment, to be brought into plant glass houses, are disinfected, except where the experimental organisms are microorganisms. Under such circumstances, treatment is at the dis cre tion of the project supervisor.

2. Pots, troughs and other plant containers are underlain with a network of watertight trays which empty directly into the drainage system.

3. Plant glasshouses and antechambers are fumigated against microorganisms after each session, and immediately against arthropods upon any sign of infestation.

4. Wastewater collected in the central drainage tanks are treated to kill viable material (e.g. microorganisms, arthropod and plant materials) before discharge.

5. In the event of a power failure, entry into the glasshouse is prohibited until the situation is abated, and power is restored.

Appendix 13

Requirements for Biosafety Level C1A in Animal Houses

Biosafety levels C1A and C2A are the two prevalent classes or grades of physical containment afforded by animal houses.* Al though emphasis is on the containment of experimental animals, the extent of concern encompasses the need for containment of other regulated material involved as well (e.g. donor DNA, biological vectors, tissue cultures). As with physical containment in laboratories, con tain ment in animal houses is achieved through the adoption of proper operating procedures, containment equipment and room design.

Biosafety level C1A provides a moderate level of animal containment and embodies the rudimentary precautions/constraints for all animal house work, including the engineering

of transgenic and infectious species.

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* Equivalent or higher levels of animal containment have no formal designation but may be archived through alternative selection of room design, equipment and operational routines or through the adoptation of supplementary precautions.

Design and Equipment

1. The entire animal house is constructed of impervious or impermeable surfaces, which may be easily cleaned and disinfected.

2. Sills under doorways and egresses are sealed or screened to deter rodents.

3. Windows and other openings along walls and ceilings (e.g. vents) are fitted with fine screens (standard: 60 x 40 swg mesh, 51% free area).

4. Drainage conduits are engineered to deter rodents, insects and other pests. Floor drains are protected with liquid disinfectant or water traps.

5. An antechamber is required at each access, if the animal house is free-standing. The innermost door should open inwards and be self-closing. A closet, for protective clothing and apparel, is provided within. (Please Note: For some reason or other, antechambers may still be required even where access to the animal house is through a large-scale containment facility)

6. Doors to animal rooms open inwards and are self-closing.

7. Biological safety cabinets are provided, as specified for containment level C1. (Appendix 7)

8. Tanks, aquariums and other rearing vessels, for invertebrates and aquatic vertebrates, are outfitted with mechanisms to guard the water supply and discharge system against experimental organisms and their gametes.

9. The entrance to the animal house is labeled with an official sign designating the certified biosafety level (available from the IBC, pending certification), with the universal biohazard symbol and, when work is in progress, with a notice detailing the entry requirements and procedures. Names and contacts (e.g. postal address, telephone and pager number) of the responsible authorities should be clearly indicated.

10. Signs are posted within the animal house, outlining the appropriate operating procedures, contingency plans and instructions for upkeep and maintenance.

Please Note: Specifications for animal rooms, cages, tanks, aquariums and the like will vary with the species involved. Investigators are strongly encouraged to consult with the NBC in preparing appropriate caging or housing requirements.

Operational Routines

1. When work is in progress and when experimental animals are 'uncaged,' all doors to the animal house are closed and entrance is restricted at the discretion of the project supervisor. On the whole, entry is reserved for those whose presence is warranted in

program or support activities. Entry requirements are binding and IBC authorization must be obtained for access.

2. All doors to the animal house are kept locked during off-hours, to prevent inadvertent access.

3. Personnel sign a logbook, indicating the date and time of each entry and exit.

4. In the antechamber, before entering the animal house, personnel don special protective clothing (e.g. jumpsuits), and put on head covers, overshoes, gloves, and pads as necessary. Particular care should be taken to avoid being bitten, clawed or scratched. Molded surgical masks and respirators should likewise be considered, where there is concern over the inhalation of aerosols.

5. Protective clothing and apparel are removed upon leaving the work area and kept in a closet provided in the antechamber.

6. Eating, drinking, smoking and applying cosmetics are all prohibited.

7. Food and drinks, for human consumption, may not be stored in the work area at any time.

8. All procedures (particularly, change of bedding material and washing or hosing down cages and pens) are performed cautiously to minimize the creation of aerosols. Whenever possible, those pro ce dures which tend to generate aerosols should be done in biological safety cabinets.

9. Personnel wash their hands with liquid soap and warm water after handling experimental organisms and cultures, and before leav ing the animal house.

10. Instruments used in culture work or with contaminated material are to be disinfected after use, if the instruments are not readily steam sterilized. For glassware, a hypochlorite solution made up with 5000 ppm of chlorine (dilute household bleach 1:8 and allow to react for at least 30 minutes), prepared daily, provides a suitable disinfectant.

11. Floors, work benches and surfaces are decontaminated with a suitable disinfectant after each session and immediately after any spill of viable material. Other than the basic hypochlorite solution, dis in fec tants must be prepared and used according to the manufacturer's instructions.

12. Animal cages and pens are cleaned regularly and decontaminated after use. Bedding material and animal wastes from cages and pens are inactivated or sterilized, along with other biological wastes (e.g. animal carcasses, tissue cultures), before disposal.

13. Ideally, modified and 'pure' animals are kept apart and in separate cages, to prevent escape of transgenes.

14. Whenever possible, procedures which may provoke experimental animals should be avoided.

15. Regulated materials are packaged within securely-sealed double containment units (section 5.1) before being removed from the animal house to rooms and facilities elsewhere. Live animals and tissues shall only be delivered to a certified containment

facility. Transfer to other institutions must be approved by the supervising IBC. Containers of viable or contaminated material are sterilized before disposal.

16. An appropriate pest control program is in effect, as supervised by a licensed pest control operator.

17. A central register is maintained for daily activities, documenting the course of work and the housing or disposal of experimental animals.

18. Animal houses are inspected and serviced periodically. Screens, filters, ventilation and drainage systems are cleaned regularly.

Please Note: Experimental animals under genetic manipulation work, and their tissues, may not be taken and used for other purposes.

Special Procedures for Work with Invertebrates

Work with invertebrates that crawl, jump or fly should observe the following, additional criteria:

- Cages and rearing tanks are numbered and documented.
- Measures are in place to detect, trap, recapture and/or destroy escaped invertebrates. An electric insect control device may be appropriate.
- Containers of ticks, mites and the like should be kept over trays of oil.
- Flying or crawling arthropods are handled on a white, or a contrasting-colored tray, to readily detect signs of flight and escape.
- Chilling methods are adopted to иanaesthetizeн active arthropods, and lessen the risk of flight or escape.

Recognizing that many invertebrates are carriers of human pathogens (e.g. midges, mosquitoes and biting flies harbor dangerous arboviruses while soft ticks are carriers of *Borrelia* and triatomid bugs are vectors for trypanosomes), experimental species known to harbor infectious microorganisms need to be contained under a level that is appropriate to the severity of any diseases borne.

Appendix 14

Requirements for Biosafety Level C2A in Animal Houses

Biosafety level C2A provides a one-step higher level of animal containment and incorporates much of the technicalities specified for laboratory containment level C2. Please refer to Appendix 8, Re quire ments for Containment Level C2, in reviewing the constraints and criteria described below.

Design and Equipment (all of biosafety level C1A, and the following...)

1. The animal house is isolated from and does not open onto public walkways. Controlled areas are physically separated from oth er areas of the animal facility.

2. Doors to the animal house are fitted with a 'fire-escape lock' which requires a special

key for entry, but opens outward freely from the inside.

3. An airlock is provided at each access, as specified for containment level C2. (Appendix 8)

4. Ventilation designs allow the animal house to operate at a requisite room pressure of 50 pascals below external air pressure, when all doors are closed; during entry and exit through airlocks, internal air pressure shall remain at least 25 pascals below external pressure. In de pen dent room exhaust fans and adjustable replacement-air apertures support room pressure control.

5. Air supply and exhaust ducts are fitted with HEPA filters. Filters should be of medium grade, with a minimum arrestance efficiency of 95% against all particles above 5 micrometers. An initial coarse or roughing filter should be considered, where there is concern over the clogging of exhaust filters with animal hair, downing, shed ding and feed dust.

6. Manometers are installed to monitor air pressure differentials and a Magnehelic type differential pressure gauge is outfitted to mea sure room negative pressure. These various sensing devices set off or sound an alarm to indicate loss of room pressure control.

7. Climate control systems are in place, where exhaust ven ti la tion rates alone cannot maintain a suitable atmosphere for animal com fort/welfare. 'Split-type' air-conditioners are preferred, where supplementary cooling is required, as the circulation of external air should be avoided.

8. There is direct or close access to an autoclave.

9. Washbasins and sinks for hand washing may be operated by foot, by elbow or automatically. Disinfecting footbaths are provided before the internal antechamber doors, leading into the glasshouse.

10. The finishes on work benches and furniture are impervious and may be easily cleaned and disinfected.

11. The animal house can be sealed-off to allow for decontamination of the entire room with formaldehyde gas. Systems are in place to render the fumes safe for discharge into the atmosphere.

Operational Routines
(all of biosafety level C1A, and the following...)

1. Work clothing is decontaminated by autoclaving, and laundered regularly, after each session.

2. Drainage exits on the floor are plugged with an airtight stop per, when experimental animals are present.

3. Cages and pens are autoclaved, in ready for cleaning and washing.

4. Work surfaces and biological safety cabinets are decontaminated with formaldehyde gas after each session and after major spills of viable material.

5. All waste material is autoclaved prior to disposal.

6. Live experimental animals must not leave the controlled areas.

Please Note: No other work must be done simultaneously with work requiring C2A levels of animal containment.

Appendix 15

Statutes on the Import of Whole Organisms

1. Infectious/Communicable Diseases Act, 1990

2. Order of the Department of Livestock Development, ??? 161/2531 (1988), Re: Movement of Animals and Animal Carcasses within the Kingdom

3. Diseases and Animal Toxins Act, 1982

4. Plant Quarantine Act, 1964

Appendix 16

Constitution of the National Biosafety Committee

Order of the National Science and Technology Development Council
4/2538 (1995)
Re: Constituting the National Biosafety Committee

Following the 2nd deliberation of the National Science and Technology Development Council for the year 1995, on March 8, 1995 (the meeting of 2/2538), a resolution was in favor, passed for the con sti tu tion of a National Biosafety Committee; as such

To actualize the above resolution, by virtue of the powers vested in the council under clause 10 of the Science and Technology Development Act of 1992, the National Science and Technology Development Council does hereby order the constitution of the National Biosafety Committee, consisting of the following individuals:

Membership:

1. Bunpot Napompeth,	Chairman
2. Sutat Sriwatanapongse,	Deputy Chairman
3. Sakol Panyim,	Member
4. Sakarindr Bhumiratana,	Member
5. Jinda Jan-Orn,	Member
6. Pichit Tosukhowong,	Member
7. Supat Attathom,	Member
8. Patanan Sangkatawat,	Member
9. Wichai Kositaratana,	Member
10. Skorn Mongkolsuk,	Member

11. Poonsook Atthasampunna,	Member
12. Representative from Office of Environmental Policy and Planning,	Ex officio member
13. Director of Food Control Division, The Food and Drug Administration or Representative,	Ex officio member
14. Director of Agricultural Regulatory Division, Department of Agriculture or Representative,	Ex officio member
15. Director of Biological Products Division, Department of Medical Sciences or Representative,	Ex officio member
16. Director of Disease Control Division, Department of Livestock Development or Representative,	Ex officio member
17. Deputy Director of the National Center for Genetic Engineering and Biotechnology,	Ex officio member and Secretary
18. An officer of the National Center for Genetic Engineering and Biotechnology,	Ex officio member and Assistant to Secretary
19. An officer of the National Center for Genetic Engineering and Biotechnology,	Ex officio member and Assistant to Secretary

The National Biosafety Committee shall have the following authorities and functions:

A. Ensure that ambient conditions surrounding genetic manipulation work reflect and adhere to the specifications of national guide lines for the safety of personnel, the community and the environment exposed to the risks borne by the study.

B. Cooperate with the Customs Department and with other relevant state authorities overseeing the import of live organisms to formulate guidelines for the identification, inspection and regulation of transgenic species, exotic and otherwise.

C. Review and direct the bearings of research methodologies in genetic engineering.

D. Identify, characterize and assess the hazards associated with innovative genetic

manipulation techniques or research for which the risks are as yet uncertain.

E. Warn the authorities and individuals who are involved with, or who may be afflicted by genetic manipulation experiments, of potential hazards throughout the conduct of work.

F. Recommend, instruct and lend specialist technical expertise to various research institutions and regulatory agencies in setting up appropriate experimental conditions for work with specific regulated material.

G. Facilitate all levels of supervision of genetic manipulation work by establishing, and assisting other regulatory bodies in es tab lish ing pertinent codes, disciplines and guidelines for the appraisal of biohazards and the management of biosafeguards.

H. Coordinate efforts to inform and educate the public on biosafety issues and on proposed national policies.

I. Forge ties with foreign biosafety committees and relevant agencies overseas to ensure that genetic manipulation practices in Thai land address international biosafety concerns and observe universal codes of conduct.

The Term of the membership is two years.

All told, to be effected henceforth and cancel the Order of the National Science and Technology Development Council 1/2536 (1993), dated 22 January 1993 of Constituting the National Biosafety Committee.

Done on the 23rd of March, 1995

(Mr. Suwaj Liptapallop)

Minister of Science, Technology
and Environment; and
Chairperson of the National Science and
Technology Development Council

Order of the National Science and Technology Development Council
1/2536 (1993)
Re: Constituting the National Biosafety Committee

Following the 9th deliberation of the National Science and Technology Development Council for the year 1992, on December 9, 1992 (the meeting of 9/2535), a resolution was in favor, passed for the constitution of a National Biosafety Committee; as such

To actualize the above resolution, by virtue of the powers vested in the council under clause 10 of the Science and Technology Development Act of 1992, the National Science and Technology Development Council does hereby order the constitution of the National Biosafety Committee, consisting of the following individuals:

Membership:

1. Bunpot Napompeth	Chairman
2. Poonsook Atthasampunna	Member

3. Sakol Panyim	Member
4. Jinda Jan-Orn	Member
5. Pichit Tosukhowong	Member
6. Supat Attathom	Member
7. Patanan Sangkatawat	Member
8. Wilai Noonpakdee	Member
9. Skorn Mongkolsuk	Member
10. Representative from Office of Environmental Policy and Planning	MemberMember
11. Director of Food Control Division, The Food and Drug Administration or Representative	Member
12. Director of Agricultural Regulatory Division, Department of Agriculture	Member
13. Director of Biological Products Division, Department of Medical Sciences	Member
14. Director of Disease Control Division, Department of Livestock Development	Member
I5. Director of the National Center for Genetic Engineering and Biotechnology	Ex officio member and Secretary
16. Deputy Director of the National Center for Genetic Engineering and Biotechnology	Ex officio member Assistant to Secretary

The National Biosafety Committee shall have the following authorities and functions:

A. Ensure that ambient conditions surrounding genetic manipulation work reflect and adhere to the specifications of national guidelines for the safety of personnel, the community and the environment exposed to the risks borne by the study.

B. Cooperate with the Customs Department and with other relevant state authorities overseeing the import of live organisms to formulate guidelines for the identification, inspection and regulation of transgenic species, exotic and otherwise.

C. Review and direct the bearings of research methodologies in genetic engineering

D. Identify, characterize and assess the hazards associated with innovative genetic manipulation techniques or research for which the risks are as yet uncertain.

E. Warn the authorities and individuals who are involved with, or who may be afflicted by genetic manipulation experiments, of potential hazards throughout the conduct of work.

F. Recommend, instruct and lend specialist technical expertise to various research institutions and regulatory agencies in setting up appropriate experimental conditions for work with specific regulated material.

G. Facilitate all levels of supervision of genetic manipulation work by establishing, and assisting other regulatory bodies in establishing pertinent codes, disciplines and guidelines for the appraisal of biohazards and the management of biosafeguards.

H. Coordinate efforts to inform and educate the public on biosafety issues and on proposed national policies.

I. Forge ties with foreign biosafety committees and relevant agencies overseas to ensure that genetic manipulation practices in Thai land address international biosafety concerns and observe universal codes of conduct.

All told, to be effected henceforth.

Done on the 22nd of January, 1993

(Mr. Phisan Moonlasartsathorn)

Minister of Science, Technology
and Environment; and
Chairperson of the National Science and
Technology Development Council

Appendix 17

Constitution of the Ad Hoc Biosafety Sub-Committee

Order of the National Science and Technology Development Council
13/2535 (1992)

Re: Constituting the Ad Hoc Biosafety Sub-Committee

Following the 5th deliberation of the National Science and Technology Development Council for the year 1992, on April 9, 1992 (the meeting of 5/2535), a resolution was in favor, passed for the constitution of an Ad Hoc Biosafety Sub-Committee; as such

To actualize the above resolution, by virtue of the powers vested in the council under clause 10 of the Science and Technology Development Act of 1992, the National Science and Technology Development Council does hereby order the constitution of the Ad Hoc Biosafety Sub-Committee, consisting of the following individuals:

Membership:

1. Bunpot Napompeth	Chairman
2. Sakarindr Bhumiratana	Vice Chairman
3. Pornchai Matangkasombut	Advisor
4. Poonsook Atthasampunna	Member
5. Sakol Panyim	Member

6. Jinda Jan-Orn	Member
7. Sonthi Vannasaeng	Member
8. Pichit Tosukhowong	Member
9. Supat Attathom	Member
10. Patanan Sangkatawat	Member
11. Wilai Noonpakdee	Member
12. An officer of the National Center for Genetic Engineering and Biotechnology	Ex officio member and Secretary
13. An officer of the National Center for Genetic Engineering and Biotechnology	Ex officio member and Assistant Secretary

All told, to be effected henceforth.
Done on the 10th of April, 1992

(Mr. Sanga Sabhasri)

Minister of Science, Technology
and Environment; and
Chairperson of the National Science and
Technology Development Council