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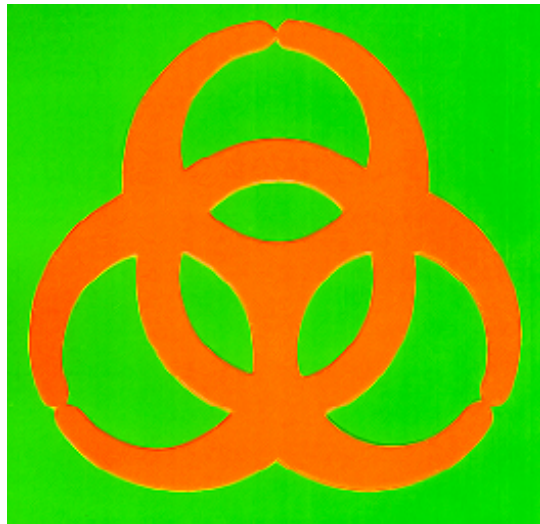
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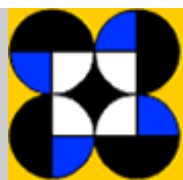
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New methods of genetic manipulation of plants, microorganisms and animals offer many possibilities for improved quality and production. Many new products in agriculture, aquaculture, health, industry and environmental remediation have emerged via new biotechnological processes. These techniques are available now but must be applied according to a number of principles to ensure safe and effective use. These principles will enable the safe application of biotechnology and other biological research and development work in a quick, responsible and orderly manner.



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NCBP Preface

New methods of genetic manipulation of plants, microorganisms and animals offer many possibilities for improved quality and production. Many new products in agriculture, aquaculture, health, industry and environmental remediation have emerged via new biotechnological processes. These techniques are available now but must be applied according to a number of principles to ensure safe and effective use. These principles will enable the safe application of biotechnology and other biological research and development work in a quick, responsible and orderly manner.

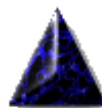
Thus, on 15 October 1990, President Corazon C. Aquino issued Executive Order No. 430 instituting the National Committee on Biosafety of the Philippines (NCBP). One of NCBP's tasks is the formulation of national policies and guidelines on biosafety.

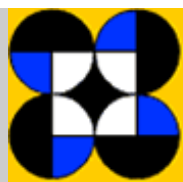
These biosafety guidelines originated from the report in 1987 of the joint Ad-hoc committee on biosafety constituted by the University of the Philippines at Los Banos, the International Rice Research Institute and the Department of Agriculture. Using as reference the biosafety guidelines of Australia, United States and Japan, the Ad-hoc committee formulated guidelines applicable to work (i.e. research, development, production/manufacture) involving biological materials especially where genetic manipulation is involved or where there is introduction of exotic or imported plants, microorganisms or animals.

This UPLB-IRRI-DA proposal underwent several rounds of consultations with both the private and public sector under the auspices of the National Academy of Science and Technology. The resulting draft was then submitted to the Department of Science and Technology with a recommendation that the NCBP be established.

Upon the constitution of the NCBP, the draft biosafety guidelines were subjected to another round of scrutiny before approval.

These biosafety guidelines (first edition) are therefore adopted for implementation in the Philippines.





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Definition of Terms

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

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

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

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

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

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

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

Genetic engineering - the genetic modification of organisms by any process.

Host-vector (HV) system - a microbial strain (host) and its compatible DNA carrier(s) (vector). The host may be a strain of the bacterium *Escherichia coli* or *Bacillus subtilis*, the yeast *Saccharomyces cerevisiae* or other such organisms that have been genetically manipulated to allow the multiplication and expression of the vector. The vector may be a plasmid, a bacteriophage or a virus, and other carriers of genetic materials all designed to carry readily selectable marker(s) and unique restriction sites for inserting DNA segments.

Introduce (or introduction) - to bring into or in-transit through the Philippines, to release into the environment, or to cause inter-island movement.

Move (moving, movement) - to ship, offer for shipment, offer for entry, import, receive for transportation, carry, or otherwise transport or allow to be transported into, through, or within the Philippines.

Organism - any active, infective, or dormant stage or life form of an entity characterized as living, including plants, bacteria, fungi, mycoplasmas, mycoplasma-like entities, vertebrate and invertebrate animals, as well as entities such as viroids, viruses, or any living entity related thereto.

Pathogen - a disease-causing organism.

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

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

Plant - any living stage or form of any member of the plant kingdom including, but not limited to, eukaryotic algae, mosses, club mosses, ferns, angiosperms, gymnosperms, and lichens (which contain algae) including any parts (e.g. pollen, seeds, cells, tubers, stems) thereof, and any cellular components (e.g. plasmids, ribosomes, etc.) thereof.

Pest - any living stage (including active and dormant forms) of insects, mites, nematodes, slugs, snails, protozoa, or other animals, bacteria, fungi, other parasitic plants or reproductive parts thereof; viruses; other plants and animals that can damage aquatic and terrestrial ecosystems; or any infectious agents or substances which can directly or indirectly injure or cause disease or damage in or to humans, plants or animals or any processed, manufactured, or other products of plants or animals.

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

Phage - eating or destroying characteristic of a bacterial virus.

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

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

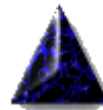
Recombinant DNA - a DNA molecule into which a foreign DNA has been inserted.

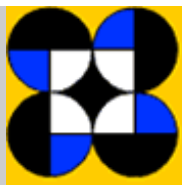
Regulated article or material - any organism which has been altered or produced through genetic engineering, if the donor organism, recipient organism, or vector or vector agent belongs to any genera or taxa designated in Appendix 3 and/or meets the definition of pest, or pathogen, or is an unclassified organism and/or an organism whose classification is unknown; or any product which contains such an organism, or any other organism or product altered or produced through genetic engineering which the appropriate authority determines, or has reason to believe, is a pest or pathogen. Excluded are microorganisms and products which are not pests or pathogens, that have resulted from genetic manipulations in which all donor and recipient organisms and materials are well characterize and innocuous.

Release into the environment - the use of a regulated material outside the physical confinement found in a laboratory, a contained greenhouse, a fermenter or other contained structure.

Responsible individual - someone who has control and who will maintain control over the introduction of the regulated article and will assure that all conditions contained and requirements set in the permit are complied with. The responsible individual shall be a resident of the Philippines or may be a designate representative who is a resident of the Philippines.

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





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National & Institutional Biosafety Committees

The National Committee on Biosafety of the Philippines (NCBP) has been established to oversee the compliance with policies and guidelines in all institutions--public or private--as well as coordinate with the appropriate national bodies that have regulatory powers over any violations. Institutional Biosafety Committees (IBC) will be established to create organizational awareness on biosafety to ensure compliance with biosafety guidelines. The institution shall appoint Biological Safety Officer(s) (BSO) if it engages in research, production or introduction activities involving genetic engineering.

A. National Committee on Biosafety of the Philippines (NCBP)

1. Composition of the NCBP

1.1 The NCBP shall be composed of the following: Chairperson - DOST Undersecretary for R & D Members 1 biological scientist 1 environmental scientist 1 physical scientist 1 social scientist 2 respected members of the community 1 representative each from the DA, DENR, and DOH to be designated by the respective Heads Offices

1.2 Procedures for appointment of the NCBP members:

1.2.1 Members of the Committee who are not connected with the organization to be regulated shall be appointed from different sectors by the President of the Republic of the Philippines, upon the recommendation of the Secretary, Department of Science and Technology.

1.3 The members of the NCBP shall have the following terms of office:

1.3.1 The term of office of the Chairperson shall be co-terminus with his/her appointment as Undersecretary for R & D; and,

1.3.2 All members, excluding the Chairperson, shall serve for a term of three (3) years, renewable for circumstances; Provided that, the members representing DA, DENR and DOH shall hold the positions for the duration of the term of their appointments in such agencies.

1.4 Deliberations of the Committee

1.4.1 No members of the Committee shall vote in deliberations affecting projects that one serves in as study/project/program leader, consultant, director, owner of biotechnology projects and/or ventures falling within purview of the Committee's concerns.

1.5 The members of the NCBP should have the following qualifications:

1.5.1 Filipino citizens of unquestionable integrity who reside permanently in the Philippines;

1.5.2 The four (4) scientist-members shall possess a minimum of seven (7) years of collegiate and postcollegiate training (degree and/or non-degree) in their respective fields.

2. Powers and Functions of the NCBP The NCBP shall have the following powers and functions:

2.1 Identity and evaluate potential hazards involved in initiating genetic engineering experiments or the introduction of new species and genetically modified organism and recommend measures to minimize risks;

2.2 Formulate, review, or amend national policies and guidelines on biosafety, such as, the safe conduct of work on genetic engineering, pests, and their genetic materials, for the protection of public health, environment, and personnel; and supervise the implementation thereof;

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

2.4 Develop working arrangements with the government quarantine services and institutions in the evaluation, monitoring, and review of projects vis-a-vis adherence to national policies and guidelines on biosafety;

2.5 Assist in the development of technical expertise, facilities, and other resources for quarantine services and risk assessments;

2.6 Recommend the development and promotion of research programs to establish risk assessment protocols and assessment of long-term environmental effects of biological research covered by these guidelines;

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

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

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

2.10 Call upon the assistance of any agency, department, office, bureau including government-owned and/or controlled corporations.

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

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

3. Meetings of the NCBP The Committee shall meet once a month and may hold special meetings to consider urgent matters at the decision of the Chairperson.

4. Quorum Six (6) members including the Chairperson shall constitute a quorum for the transaction of business.

5. Adoption of Resolutions, Guidelines or Policies The affirmative vote of at least six (6) members shall be necessary for the adoption of resolutions, guidelines, or policies.

6. Compensation of NCBP Members Members shall be entitled to an appropriate per diem which shall be determined by the Secretary of the DOST.

B. The Institutional Biosafety Committee (IBC) All institutions engaged in genetic engineering and/or potentially hazardous biological and/or genetic engineering work are required to have an Institutional Biosafety Committee (IBC) which will evaluate and monitor the biosafety aspects of their biological research. Where an institution intends to become involved in planned field release research, members of the IBC should collectively have the range of expertise necessary to supervise and assess this.

1. Composition of IBC

1.1 The Committee shall be composed of five(5) members with expertise in genetic engineering or pests, or who have the capability to assess the safety of

research. At least two (2) members shall not be affiliated with the institution (apart from their membership with the IBC) and shall represent the interest of the surrounding community with respect to health and protection of the environment;

1.2 IBC may have consultants-on-call who are knowledgeable on the commitments and policies of the institution; applicable laws, standards of professional conduct and practice; community attitudes; and the environment.

2. Functions of the IBC It shall be the function of the IBC to:

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

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

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

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

2.2 Notify the projects chief/proponent/investigator about the results of the review;

2.3 Review monthly, the work with potential risks being conducted at the institution to ensure that requirements of the Guidelines are being fulfilled;

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

2.5 Report immediately to the appropriate official in the concerned organization and to the NCBP, any significant problems with or violations of the Guidelines and any significant research-related accidents or illnesses;

2.6 Perform such other functions as may be delegated by the NCBP.

3. Meeting of the IBC The Committee shall meet every quarter and may hold special meetings to consider urgent matters upon the decision of the Chairperson.

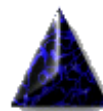
4. Quorum Three (3) members, with at least one being a community representative, shall constitute a quorum for the transaction of business.

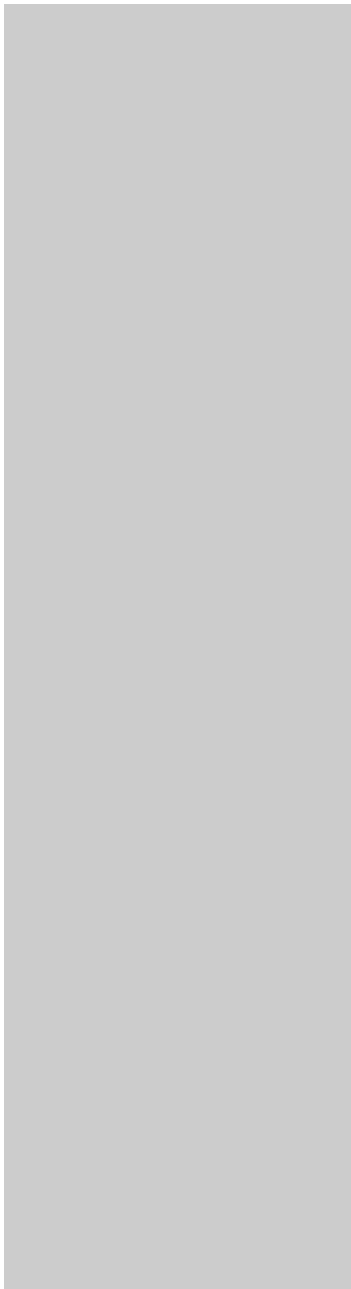
C. The Biological Safety Officer(s)(BSO)

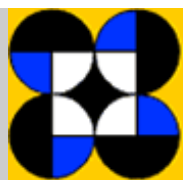
1. The BSO(s) shall be designated by the institution engaged in research, production and introduction activities involving genetic engineering. More than one BSO may be appointed, depending on the needs of the institution.

2. It shall be the duty of the BSO to monitor compliance with the Guidelines.

3. The BSO may or may not be a member of the IBC.







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EVALUATION OF PROJECT RESEARCH PROPOSALS IN POTENTIALLY HAZARDOUS BIOLOGICAL WORK

1. IBC Procedures

1.1 Before any experiment on organisms is undertaken, the principal investigator or project manager should prepare a proposal according to the format set by the NCBP. The Proposal should respond to all items indicated in the Procedures for Evaluation.

1.2 The Cover Sheet, which appears in Appendix 2, should be attached to the proposal. The Cover sheet requires the institution to identify the government or private agency, if any, which will be responsible for the over-all assessment of the project. The research laboratory and its location must be certified by the IBC/NCBP as having complied with biosafety standards and requirements. Its operation should be monitored monthly by the IBC.

1.3 This proposal should then be submitted to the IBC. The IBC should assess the project and then send the original proposal with its own evaluation, to the NCBP for assessment. Specific queries of the IBC should be included in this evaluation.

1.4 Genetic manipulation of organisms should be allowed only if the ultimate objective is for the welfare of humanity and the natural environment and only if it has been clearly demonstrated that there is no existing or foreseeable alternative approaches to servicing the welfare of humanity and the natural environment. The use of domestic animals in tests involving products of genetic engineering is subject to approval of IBC and NCBP.

1.5 Upon receipt of the NCBP's assessment, the IBC, in consultation with any regulatory agency, should arrange to make changes recommended by the NCBP, if there are any.

1.6 The IBC should monitor the progress of the work and immediately report to the NCBP any significant, unforeseen occurrences regarding work. Unless otherwise required, the IBC should also advise the NCBP annually on the progress of the work in so far as safety and environmental protection are concerned.

1.7 If institutions provide the IBC/NCBP with information that is not in the public domain and they wish to restrict access, the principal investigator/project manager should mark the relevant pages "commercial-in-confidence".

1.8. Members of the IBC/NCBP will sign deeds of confidentiality binding them not to divulge or use commercial-in-confidence information.

2. The NCBP Procedures

2.1 The NCBP will require up to eight (8) weeks from receipt of the proposal to conduct its biosafety assessment and to respond to the IBC unless additional information is needed from the proponent requiring an extension of the assessment period. Assessment of risk should be based on the characteristics of the biological product and on the process by which it was obtained.

2.2 Upon receipt of proposal, the NCBP will form a working group, members of which may be drawn from the NCBP itself, who will assess the proposal based on the Procedures for Evaluation. This working group may seek additional requirements either in elaboration of the proposal or on new issues that may arise from an examination of the proposal. The working group shall submit its recommendations to the NCBP.

2.3 The assessment of the NCBP will be sent, for appropriate action, to the institution's IBC and to the regulatory agency listed in question 8 of the Cover Sheet.

2.4 The NCBP shall furnish the relevant government agencies with a list of all projects submitted for the year.

2.5 In the case of NCBP members whose expertise is needed in the assessment of a proposal, the expertise of that NCBP member may be tapped by the IBC making the assessment.

3. Procedures for Evaluation

The proposal should include the following:

- 3.1 Title
- 3.2 Program/Project/Study Leader(s)
- 3.3 Name of Institution
- 3.4 Cooperating Institution(s)
- 3.5 Objectives of the Research
- 3.6 Materials and Methods
 - 3.6.1 Degree of Genetic Manipulation
 - 3.6.2 Methodology/Protocol (including timetable of activities)
 - 3.6.3 Location of Experiment
 - 3.6.4 Characteristics of the Organisms Research Organisms : (Plants, Animals, Microorganisms)
- a. Local strains
 - a.1 collected within the region (mention exact location);
 - a.2 collected from other regions - origin of strains.
- b. Imported strains (indicate point of origin)
 - b.1 not present in the country;
 - b.2 present but of restricted distribution in the country;
 - b.3 widely distributed in the country.
- c. Genetically modified strains
- d. Auto-Ecology
 - 3.6.5 For Experiments Involving rDNA Plant
 - 3.6.5.1 Host Organism for rDNA experiment
 - a. Nomenclature;
 - b. The state of cultivation or distribution in nature;
 - c. Reproductive cycle;
 - d. Possibility of natural crossing to related species;
 - e. Producibility of toxic substances;
 - f. Weediness/effect on environment (soil, water, etc.).
 - 3.6.5.2 Characteristics of donor DNA
 - a. Constitution and origin;
 - b. Functions of the target gene;
 - c. Characteristics of donor organisms
 - c.1 Taxonomy, identification, source culture;
 - c.2 The state of cultivation, distribution in nature;
 - c.3 Reproductive cycle;
 - c.4 Possibility of natural crossing to related species;
 - c.5 Producibility of toxic substances;
 - c.6 Weediness.
 - c.7 Auto-Ecology
 - 3.6.5.3 Name, designation, origin and characteristics of the vector
 - 3.6.5.4 Construction method of the rDNA organisms (where applicable):
 - a. Structure and construction method of the recombinant molecule;
 - b. Method to introduce target genes into recipient cells -
 - 3.6.5.5 Characteristics of rDNA organisms (where applicable):
 - a. Comparison with recipient organisms;
 - b. Localization, copy number and stability of the target gene in recipient cell;
 - c. Stability of the introduced gene expression.
 - 3.6.5.6 Other important points obtained in the rDNA experiment to develop the rDNA organisms
 - 3.6.5.7 Characteristics of the plant for breeding material;
 - a. Form of heredity of the target phenotype;
 - b. Genetic stability of the target phenotype;
 - c. Reproductive cycle;
 - d. Natural crossing possibility to related species;
 - e. Producibility of toxic substances;
 - f. Weediness/effect on environment (soil, water, etc.).
 - g. Auto-Ecology
 - 3.6.5.8 Object of the breeding

3.6.5.9 Other important points obtained in the rDNA experiment or in the process of growing into breeding material

3.6.6.0 For Experiments Involving rDNA Microorganisms

3.6.6.1 Characteristics of the recipient organisms:

- a. Nomenclature (scientific name and strain);
- b. Genetic properties (Characteristics):
 - b.1 History of prior genetic manipulation, if any;
 - b.2 Factors which might limit the reproduction, growth and survival of the recipient organisms; stability of genetic traits.
- c. Characteristics and stability of plasmids, phages, viruses, in the recipient organisms;
- d. Reproductive cycle (sexual or asexual).

3.6.6.2 Pathogenicity, active compounds:

- a. Pathogenicity (details and availability of appropriate prophylaxis and therapies, if any);
- b. Producibility of biological active compounds;
- c. Adventitious agents;
- d. Prior reports of a history of industrial use, if any.

3.6.6.3. Characteristics of the related strain of recipient organisms:

- a. Natural habitat and geographic distribution;
- b. Genetic traits
 - b.1 Characteristics and stability of plasmids, phages, viruses, in the recipient organisms;
 - b.2 Crossing possibility.
- c. Pathogenicity, active compounds;
 - c.1 Pathogenicity (details and availability of appropriate prophylaxis and therapies, if any);
 - c.2 Producibility of biological active compounds;
- d. Prior reports of a history of industrial use, if any.

3.6.6.4. Characteristics of donor DNA

- a. Construction origin;
- b. Functions of the objective genes;
- c. Properties of DNA donor;
 - c.1 Nomenclature (scientific name and strain);
 - c.2 Pathogenicity, producibility of biological active compounds.

3.6.6.5 Name, designation, origin, characteristics of the vector

3.6.6.6 Construction method of the rDNA organisms

- a. Structure and construction method of the recombinant molecules;
- b. Method to introduce target genes into recipient cells.

3.6.6.7 Characteristics of the rDNA organisms

- a. Comparison with recipient organisms
 - a.1 Characteristics with respect to survival, growth and reproduction;
 - a.2 Crossing possibility;
 - a.3 Pathogenicity, infectivity;
 - a.4 Producibility of biological active compounds.
- b. Target gene
 - b.1 Localization, copy number and stability of the target gene in recipient cells;
 - b.2 Stability of the introduced gene expression;
 - b.3 Genetical manipulations applied to already modified rDNA organisms;
 - b.4 Method to restrict the multiplying ability in open environment.

3.6.6.8 Other important remarks obtained in the rDNA experiment or during the preliminary application in the controlled model environment.

3.6.7 Other Considerations in Assessing Characteristic of Organisms:

3.6.7.1 Known potential of natural variants to cause epidemics (survival rates, reproduction, dispersal, etc.)

3.6.7.2 Known potential to cause losses (plant part affected, phenological stage affected, etc.)

3.6.7.3 Known potential hosts and their economic or social importance

3.6.7.4 Known natural ability to evolve

3.6.7.5 Known carriers of organism and abundance

3.6.7.6 Provide the required information below:

3.6.7.7 Epidemiological factors

- a. mode of spread including vectors and other transport hosts;
- b. environmental conditions needed for epidemics;
- c. history of epidemic.

3.6.7.8 Laboratory Environment

- a. containment capabilities of laboratory;
- b. sterilization procedures;
- c. personnel awareness of biosafety procedures;
- d. past history of biosafety in laboratory;
- e. labeling/designation of "risk" areas;
- f. decontamination facilities;
- g. "biosafeness" of equipment.

3.6.8 Host Range

3.6.9 Additional Considerations or End Uses Specific to Particular Organisms

3.6.9.1 Microorganisms

a. Live Vaccines

a.1 Specify/give the identification characteristics or markers, the growth requirements, and the genetic modification of the vaccine strain of the organism.

a.2 Specify the proposed dose rate(s). Give the period when the vaccine organism can be detected in the vaccinated animals and their excretions.

a.3 Indicate if the vaccine organisms spread from vaccinated to in-contact, non-vaccinated animals of the same or other animal species. If so, state the mechanisms and frequency.

a.4 Give the vaccine strain's frequency of reversion to wild type characteristics.

a.5 For pen-trials, specify arrangements proposed for disposal of waste containing any vaccine organisms and of the vaccinated animals at the conclusion of the trial.

a.6 Give the clinical effects of the vaccine organism target and non-target species in the test area and surrounding environment.

a.7 Specify the level and duration of immunity produced in the target species.

a.8 State challenge or other tests using virulent field strains to be carried out on vaccinated animals.

a.9 Indicate the probability of the host vaccine organism being used in other human or animal vaccines. Specify if the use of this vaccine precludes the future use of the host vaccine organism for immunization purposes.

b. Microorganisms on Soil/Water Associated with Plants

b.1 Specify the survival and reproduction characteristics of the organism in the rhizosphere of the plant species of interest and other plant species in the test site and surrounding environment.

b.2 Give the effects on organisms likely to be in the test area which are known to be beneficial to plants (e.g. Rhizobium, Frankia and mycorrhizal fungi).

c. Microorganisms to be used for biological control
c.1 State the effects that the unmodified and modified organism have on the biological control target, the plant or animal being protected and non-target species (including humans) in the test area and surrounding environment. State, in particular, if there are any growth or quality reductions in the protected organism.

3.6.9.2 Animals

a. Domesticated or farmed animals (terrestrial, aquatic)

a.1 Indicate the desirable effects expected to result from the use of the modified animal (e.g. improved reproduction, weight gain, disease resistance and production gains).

a.2 State the undesirable effects that may result from the release of the modified organism like alteration of nutritional quality (e.g. difficult birth, fertility reduction, increased disease prevalence, tumorigenicity and production losses). Indicate if any of the likely gains are directly linked to losses in other characteristics of the species (e.g., an increased growth rate being accompanied by a decrease in wool or milk production).

a.3 Indicate if the genetic trait can be transmitted other than thru their normal reproduction (e.g., from animal to animal via virus or insect transmission).

3.6.9.3 Plants

a. Specify if any member of the genus of the modified plant is known to be harboring weeds or diseases.

b. Indicate if the experimental plot is isolated from plants of the same species, with regard to the pollination characteristics of the plant.

3.6.9.4 Plant quarantine significance

a. Provide data on the credentials of the principal investigator/researcher.

b. Provide data on any previous information on risk assessment of the organism.

c. Provide data on any markers available to track the organism if it escapes.

3.7 Release of the Organism(s) into the Environment

3.7.1 Specify what organism(s) is to be released. Give the genetic modification and the change it is expected to make to the phenotype of the released organism(s). Specify the degree of characterization of the inserted DNA. Indicate as to what organism the inserted DNA originate from. Specify the vector used in the transfer.

3.7.2 Provide data on potential hazards or deleterious effects specifically being evaluated.

3.7.3 Indicate where the release is to be made. Give the nature of the surrounding environment. Specify the facilities that exist at the site.

3.7.4 Indicate how the work is to be performed and supervised. Provide data on barriers planned to segregate the experiments from the surrounding environment. Enumerate contingency plans existing to cope with physical calamities (e.g., floods, typhoons, earthquakes, and fires).

3.7.5 Specify the arrangements for producing the modified organism in quantity.

3.7.6 Specify the arrangement for the transport of the modified organism to the site.

3.7.7 Specify the survival rates of the modified organism in the spectrum of conditions likely to be found in the release area (s) and surrounding environment(s).

3.7.8 Indicate the organism's reproduction rates in release areas and surrounding environment.

3.7.9 Indicate the capability of the organism to disperse from the release area. Indicate the dispersal mechanisms.

3.7.10 Indicate if the inserted genetic trait could be transferred to other organisms in the release site and surrounding environment; if yes, specify with what organisms and at what frequencies.

3.7.11 Indicate data available which show that the introduced genetic trait has no unforeseen deleterious effect in the long term.

3.7.12 Indicate if the modified organism is intended to modify the characteristics or abundance of other species. If yes, specify these species and the intended changes.

3.7.13 Indicate if laboratory trials have been done. Indicate the experimental results or information available which show the probable consequences (positive and negative) of the release of the modified organism, including impacts on:

- a. human and animal health;
- b. agricultural production;

- c. the target and non-target organisms in the area;
- d. the general ecology, environmental quality in the area.

3.7.14 Specify the range of consequences which has been considered (e.g., what range of species was examined for non-target effects).

3.7.15 Give unlikely but possible impacts that have been postulated. Indicate if any of these would have substantial impacts if they actually occurred. Likewise, also indicate if the release protocol minimizes or monitors these low probability risks. If so, indicate how.

3.7.16 Give the consequences of the organism remaining in the environment beyond the planned period; (Cover the same range of issues as set out in 3.7.14 and 3.7.15 above).

3.7.17 Give the methods used (and their sensitivity) to monitor the environmental impacts mentioned in points 3.7.13 to 3.7.15 above, and in particular the methods used to monitor the population of the modified target and non-target organisms.

3.7.18 Indicate the methods that will be used to control or eliminate the organism from the site and the surrounding environment should such action be required.

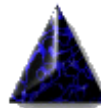
3.7.19 Indicate if it is the intention, at present, to propose a general release if the trials are successful.

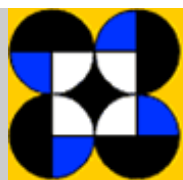
3.7.20 Provide data or any other information which the organization considers to be of assistance to the NCBP's assessment.

3.8 Experiments Involving Conventional Breeding of Plants and Animals (Selective Breeding, Mutagenesis; Protoplast, Cell and Embryo Fusion) (NOTE: These experiments shall be covered by the stipulations of existing plant and animal quarantine laws, including implementing guidelines issued by duly constituted authority.)

3.9 Experiment Involving Conventional Breeding of Microorganisms (Selective Breeding, Mutagenesis; Protoplast, Cell and Embryo Fusion)(NOTE: These experiments shall be covered by provisions of these guidelines for evaluation where appropriate, especially in 3.6.6, 3.6.7, 3.6.9.)

3.10 The proponent must demonstrate - taking into consideration scientific, ecological, economic, social and ethical concerns - that the proposed objectives of the research, as stated in Section 3.5, cannot be addressed/realized adequately and achieved by alternative approaches.





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PROCEDURES AND GUIDELINES ON THE INTRODUCTION, MOVEMENT AND FIELD RELEASES OF REGULATED MATERIALS

Procedures on the Introduction, Movement and Field Releases of Regulated Materials

1.1 Introduction of Regulated Materials

1.1.1 All persons who desire to import any organism and other regulated materials should apply for import permit from pertinent regulatory agency as required by law.

1.1.2 A written application should respond to all items listed in the guidelines for introduction of regulated materials. Application should be submitted four (4) months before importation.

1.1.3 The appropriate authorities should make any and all information on the organism available to the NCBP and should ask specific advice on any aspect of the organism or regulated material and recommendation on conditions which should be applied. Applications for importation of organisms modified by rDNA techniques should be referred to NCBP for review by the concerned agency.

1.1.4 If portions of the application contain trade secret or confidential business information, each page of the application containing such information should be marked "Commercial-in-Confidence" or "CIC Copy" by the principal investigator/project manager.

1.1.5 After review of the application and the data submitted pursuant to 1.1.2, the NCBP shall make the appropriate decision. If a permit is issued, it should specify applicable conditions for introduction of regulated materials. If the application is denied, the applicant shall be promptly informed of the reasons.

1.1.6 Any person whose application has been denied or whose permit has been withdrawn may appeal the decision in writing to the appropriate authorities within 30 days of receipt of the written notice. The appeal should clearly state all the facts and reasons to show that the permit was wrongfully withdrawn or denied. All appeals including application documents shall be referred back to NCBP for final comments and suggestions.

their guidance relative to the terms and conditions; the triplicate shall be presented to the Collector of Customs at the port of entry, and the fourth copy shall be filed with the application.

1.1.7 A person who is issued a permit should comply with conditions specified in it. Non-compliance with the conditions shall be ground for revocation of permit. It will remain revoked until such time that the specified conditions are fully complied with.

1.2 Movement of Regulated Materials

1.2.1 No organism and other regulated materials shall be introduced and moved unless guidelines on packaging and container requirements including marking and identification requirements are fully complied with.

1.2.2 All introduction of organism shall be addressed to the appropriate quarantine

officers.

1.2.3 Movement from the Quarantine to the final destination shall be under guard of a Quarantine Officer at the importer's expense. No further movement of regulated material shall be made unless authorized by appropriate authorities.

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

1.3 Field Release of Regulated Materials

1.3.1 No person is allowed to release any organism or other regulated material unless he has a permit.

1.3.2 Application for a permit and a proposed procedure for release of regulated materials into the environment duly endorsed by the IBC should be submitted to the NCBP 60 days in advance of the scheduled release. The application and research proposal should respond to all items listed in the guidelines for release.

1.3.3 The appropriate authorities, in coordination with the NCBP, shall grant a permit, if warranted. If an application is denied, appropriate explanation shall be given.

1.3.4 All persons who are granted permit to release regulated materials shall be required to submit periodic reports as specified by and to the appropriate authorities.

1.3.5 The quarantine services of the Government shall be responsible for monitoring the progress of the work and shall immediately report any significant outcome to the IBC for any remedial action.

2. Guidelines on the Introduction, Movement and Field Releases of Regulated Materials

2.1 Introduction of Regulated Materials

2.1.1 Any introduction of regulated materials should be authorized by an import permit;

2.1.2 Approval or denial of an import permit shall be based on the following guidelines for evaluation:

2.1.2.1 Responsible person or persons involved

a. Name, title, address, tele-phone number, and signature; b. Name, address, and telephone number of the person(s) who developed and/or supplied the regulated material;

2.1.2.2 Materials to be introduced

- a. Quantity of the regulated material(s) to be introduced and proposed schedule and number of introductions;
- b. All scientific, common, and trade names, and all designations necessary to identify the regulated material;
- c. Country and locality where the regulated material was collected, developed, and produced;
- d. Known potential to cause an epidemic (survival and reproductive rates, dispersal, etc.);
- e. Known potential to cause losses;
- f. Known potential hosts or alternative hosts;
- g. Known ability to evolve;
- h. Known vector of organisms;
- i. Known mode of spread and conditions for epidemic;
- j. History of epidemics;

2.1.2.3 Genetically Modified Micro-organisms

- a. Nomenclature and characteristics of donor, recipient, and vector organisms;
- b. A detailed description of the molecular biology of the systems (e.g., donor-recipient-vector) that is or will be used to produce the regulated materials;
- c. A description of the anticipated or actual expression of the altered genetic material in the regulated materials; an explanation of how that expression differs from the expression in the non-modified parental organism such as

morphological or structural characteristics, physiological activities and processes, number of copies inserted in the genetic material; the physical state of this material inside the recipient organism (integrated or extra-chromosomal), products and secretions, growth characteristics;

d. A detailed description of the processes, procedures, and safeguards that have been used or will be used in the country of origin and in the Philippines to prevent contamination, release, and dissemination in the production of the donor organism, recipient organism, vector or vector agent, regulated materials, and a constituent of each regulated material which is a product.

2.1.2.4 Others

a. A detailed description of the uses and the purpose for introducing the regulated material, including a detailed description of the proposed experimental and/or production design;

b. History of similar introductions;

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

d. A detailed description of the intended destination (including final and all intermediate destinations), and/or distribution of the regulated material (e.g., greenhouse, laboratory, or growth chamber location; field trial location; pilot project location; production, propagation, and manufacture location; proposed sale and distribution location);

e. A detailed description of the proposed procedures, processes, and safeguards that will be used to prevent escape and dissemination of the regulated material at each of the intended destinations;

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

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

2.2 Movement of Regulated Materials A person who has been issued a permit for importation shall comply with the following guidelines for movement of regulated materials:

2.2.1 Marking and Identification

2.2.1.1 General nature and quantity of the content;

2.2.1.2 Country and locality where collected, developed, manufactured, reared, cultivated, or cultured;

2.2.1.3 Name and address of shipper, owner, or person shipping or forwarding the organism;

2.2.1.4 Name, address, and telephone number of consignee;

2.2.1.5 Identifying shipper's mark and number;

2.2.1.6 Written permit number authorizing the importation. 2.2.2 Container (Please refer to Part IV, Section 5 - Container Requirements)

2.3 Field Release of Regulated Materials

2.3.1 Field release of restricted material shall be authorized by a permit from the NCBP;

2.3.2 A written application for permit and a proposed procedure for release duly endorsed by the IBC are required for issuance of a permit;

2.3.3 The application and the procedure for release shall state the following:

2.3.3.1 Aims and benefit of the planned release;

2.3.3.2 Future plans after the planned release;

2.3.3.3 Species, number, or quantity to be released;

2.3.3.4 Known or anticipated adverse effect(s) of the organism on human, animal, or plant health; agricultural production; other species; or environmental quality;

2.3.3.5 Place, nature of surrounding environment, and existing facilities where planned release is to be made;

2.3.3.6 Capability of the organism to disperse from the release area (e.g., survival and reproductive rates, dispersal mechanisms, etc.);

2.3.3.7 History of similar releases if any;

2.3.3.8 Potential hazards or deleterious effects that could be expected;

2.3.3.9 Supervision of planned release;

2.3.3.10 Contingency plans to cope with extreme conditions (e.g., typhoon);

2.3.3.11 Consequences of the organism remaining in the environment beyond the planned periods;

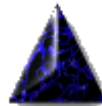
2.3.3.12 Methods used (and their sensitivities) to monitor the planned release;

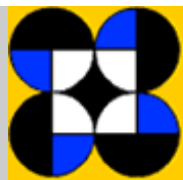
2.3.3.13 Methods used (and their efficiency) to control or eliminate the organism from the site and the surrounding environment, should circumstances so dictate.

2.3.3.14 Identification of the person who will undertake the release of the product in the field;

2.3.3.15 Other information

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





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PHYSICO - CHEMICAL AND BIOLOGICAL CONTAINMENT: PROCEDURES AND FACILITIES

1. Physical Containment

1.1 Standard Practices and Training

The first principle of containment is strict adherence to good biosafety practices. Consequently, all personnel directly or indirectly involved in experiments on rDNAs, pests, and potentially harmful microorganisms must receive adequate instruction. This shall include, at the least, instructions in aseptic techniques and in the biology of the organisms used in the experiments so that the potential biohazards can be understood and appreciated.

Any group working with regulated materials should have an emergency plan which describes the procedures to be followed if an accident contaminates personnel or the environment. Everyone should know about this plan. Physical Containment Level I (PI) must ensure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan. If a group is working with a known pathogen for which there is an effective vaccine, such vaccine should be made available to all workers.

Where serological monitoring is clearly appropriate, it should be provided. The "Laboratory Safety Monograph" and "Bio-safety in Microbiological and Biomedical Laboratories" booklets describe practices, equipment, and facilities in detail. (See References for details)

1.2 Physical Containment Levels

The objective of physical containment is to confine harmful organisms and those containing rDNA molecules and thus reduce the risk of exposure of the laboratory worker, persons outside of the laboratory, and the environment to these harmful organisms. The primary means of physical containment is achieved through proper laboratory practices and containment equipment. Special laboratory design provides a secondary means of protection against the accidental release of organisms outside the laboratory or to the environment. Special laboratory design is used primarily in facilities wherein experiments of moderate to high potential hazards are performed.

Combinations of laboratory practices, containment equipment, and special laboratory design can be made to achieve different levels of physical containment. Four levels of physical containment, which are designated as BL1, BL2, BL3, and BL4, are described in succeeding paragraphs. It should be emphasized that the descriptions and assignments of physical containment detailed below are based on existing approaches of pathogenic organisms.

It is recognized that several different combinations of laboratory practices, containment equipment, and special laboratory design may be appropriate for containment of specific research activities. The Guidelines, therefore, allow alternative selections of primary containment equipment within facilities that have been designed to provide BL3 and BL4 levels of physical containment. The selection of alternative methods of primary containment depends however, on the level of biological containment provided by the host-vector system used in the experiment. Consideration will also be given by NCBP to other combinations that achieve an equivalent level of containment.

1.3 Biosafety Level 1 (BL1)

BL1 is suitable for work involving agents of no known or minimal potential hazard to laboratory personnel and environment. The laboratory is not separated from the general traffic patterns in the building. Work is generally conducted on open bench tops. Special containment equipment is not required or generally used. Laboratory personnel work in the laboratory and are

supervised by a scientist with general training in microbiology or a related science.

1.3.1 Procedures

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

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

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

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

1.3.1.5 Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for that purpose only;

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

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

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

1.3.1.9 Contaminated materials that are to be decontaminated at a site away from the laboratory should be placed in a durable, leakproof container, which is closed before being removed from the laboratory. An insect and rodent control program is in effect, as certified by a licensed pest control officer.

1.3.2 Containment Equipment

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

1.3.3 Laboratory Facilities

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

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

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

1.3.3.4 Each laboratory contains a sink for hand-washing;

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

1.4 Biosafety Level 2 (BL2)

BL2 is similar to BL1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that (1) laboratory personnel are specifically trained to handle pathogenic agents and are directed by experienced scientists, (2) access to the laboratory is limited when work is being conducted, and (3) certain procedures in which infectious aerosols are created are conducted in biological safety cabinets or other physical containment equipment.

1.4.1 Procedures

1.4.1.1 Access to the laboratory is limited or restricted by the laboratory director when work with organisms containing rDNA molecules is in progress;

1.4.1.2 Work surfaces are decontaminated at least once a day and after any spill of viable material;

1.4.1.3 Mechanical pipetting devices are used; mouth pipetting is prohibited;

1.4.1.4 Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for that purpose only;

1.4.1.5 Persons wash their hands after handling materials involving animals and

organisms containing rDNA molecules, and when they leave the laboratory;

1.4.1.6 All procedures are performed carefully to minimize the creation of aerosols;

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

1.4.1.8 Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable, leakproof container as specified by the NCBP, which is closed before being removed from the laboratory;

1.4.1.9 The laboratory director limits access to the laboratory. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory;

1.4.1.10 The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazard and who meet specific entry requirements (e.g., immunization) can enter the laboratory or animal rooms;

1.4.1.11 When the organisms containing rDNA molecules in use in the laboratory require special provisions for entry (e.g., vaccination), a hazard warning sign incorporating the universal biohazard symbol (see Appendix 5) is posted on the access door to the laboratory work area. The hazard warning sign identifies the agent, lists the name and telephone number of the laboratory director or other responsible persons, and indicates the special requirements for entering the laboratory; An insect and rodent control program is in effect as certified by a licensed pest control operator.

1.4.1.12 Laboratory coats, gowns, smocks, or uniforms are worn by personnel while in the laboratory. Before personnel leave the laboratory for non-laboratory areas (e.g., cafeteria, library, administrative offices), this protective clothing is removed and left in the laboratory or is covered with a clean coat not used in the laboratory;

1.4.1.13 Animals not involved in the work being performed are not permitted in the laboratory;

1.4.1.14 Special care is taken to avoid skin contamination with organisms containing rDNA molecules; gloves should be worn when handling experimental animals and when skin contact with the agent is unavoidable;

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

1.4.1.16 Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of fluids containing organisms that have rDNA molecules. Extreme caution should be observed when handling needles and syringes to avoid autoinoculation and generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse;

1.4.1.17 Spills and accidents that result in overt exposures to organisms containing rDNA molecules are immediately reported to the laboratory director and the IBC. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained;

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

1.4.1.19 A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read instructions on practices and procedures and to

follow them. 1.4.2 Containment Equipment

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

1.4.2.2 Procedures with a high potential for creating aerosols are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or eggs;

1.4.2.3 High concentrations or large volumes of organisms containing rDNA molecules are used. Such materials may be centrifuged in the open laboratory if sealed heads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

1.4.3 Laboratory Facilities

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

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

1.4.3.3 Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning; 1.4.3.4 Each laboratory contains a sink for hand-washing.

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

1.4.3.6 An autoclave for decontaminating laboratory wastes is available.

1.5 Biosafety Level 3 BL3

BL3 is applicable to clinical diagnosis, teaching, research, or production facilities where work is done with indigenous or exotic agents that may cause serious or potentially lethal diseases as a result of exposure by inhalation. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents and are supervised by competent scientists who are experienced in working with these agents. All procedures involving manipulation of infectious material are conducted within biological safety cabinets or other physical containment devices. Personnel wear appropriate personal protective clothing and devices. The laboratory has special engineering and design features. It is recognized, however, that many existing facilities may not have all the facility safeguards recommended for BL3 (e.g., access zone sealed penetrations and directional airflow, etc.). In such cases, the proponent must show proof of access to BL3 facilities. Under these circumstances, acceptable safety may be achieved for routine or repetitive operations (e.g., diagnostic procedures involving the propagation of agent for identification, typing, and susceptibility testing) in laboratories where facility features satisfy BL2 recommendations, provided the recommended "standard Microbiological Practices", "Special Practices" and "Containment Equipment" for BL3 are rigorously followed. The decision to implement this modification of BL3 recommendations should be made only by the laboratory director.

1.5.1 Procedures

1.5.1.1 Work surfaces are decontaminated at least once a day and after any spill of viable material;

1.5.1.2 All contaminated liquid or solid wastes are decontaminated before disposal;

1.5.1.3 Mechanical pipetting devices are used; mouth pipetting is prohibited;

1.5.1.4 Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the work area;

1.5.1.5 Persons wash their hands after handling animals and materials involving organisms containing rDNA molecules, and when they leave the laboratory;

1.5.1.6 All procedures are performed carefully to minimize the creation of aerosols;

1.5.1.7 Persons under 16 years of age are not allowed to enter the laboratory;

1.5.1.8 If experiments involving other organisms that require lower levels of containment are to be conducted in the same laboratory concurrently with work requiring BL3 level physical containment, such experiments shall be conducted in accordance with all BL3 level practices;

1.5.1.9 Laboratory doors are kept closed when experiments are in progress;

1.5.1.10 Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable, leak-proof container, which is closed before being removed from the laboratory;

1.5.1.11 The laboratory director controls access to the laboratory and restricts access to persons whose presence are required for program or support purposes. The director has the final responsibility of assessing each circumstance and determining who may enter or work in the laboratory;

1.5.1.12 The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures enter the laboratory or animal rooms;

1.5.1.13 When organisms containing rDNA molecules or experimental animals are present in the laboratory or containment module, a hazard warning sign incorporating the universal biohazard symbol is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, the list and telephone number of the laboratory director or other responsible persons, and indicates any special requirements for entering the laboratory, such as the for immunization, respirators, or other protective measures;

1.5.1.14 All activities involving organisms containing rDNA molecules are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench;

1.5.1.15 The work surfaces of biological safety cabinets and other containment equipment are decontaminated when work with organisms containing rDNA molecules is finished. Plastic-backed paper towelling used on non-perforated work surfaces within biological safety cabinets facilities cleaning up. An insect and rodent program is in effect as certified by a licensed pest control operator.

1.5.1.16 Laboratory clothing that protects street clothing (e.g., solid front or wrap-around gowns, scrub suits, cover-alls) is worn in the laboratory. Laboratory clothing is not worn outside the laboratory, and is decontaminated before being laundered;

1.5.1.17 Special care is taken to avoid skin contact with contaminated materials; gloves should be worn when handling infected animals and when skin contact with infectious materials is unavoidable;

1.5.1.18 Molded surgical masks or respirators are worn in rooms containing experimental animals;

1.5.1.19 Animals and plants not related to the work being conducted are not permitted in the laboratory;

1.5.1.20 Laboratory animals held in a BL3 area are housed in partial-containment caging systems, such as Horsfall units (11), open cages placed in ventilated enclosures, solid-wall and -bottom cages covered by filter bonnets, or solid-wall and -bottom cages placed on holding racks equipped with ultraviolet radiation lamps and reflectors;

(NOTE: Conventional caging systems may be used provided that all personnel wear appropriate personal protective devices. These shall include, at a minimum, wrap-around gowns, head covers, gloves, shoe covers, and respirators. All personnel shall shower on exit from areas where these devices are required.)

1.5.1.21 All wastes from laboratories and animal rooms are appropriately decontaminated before disposal;

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

1.5.1.23 Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that have rDNA molecules. Extreme caution should be observed when handling needles and syringes to avoid auto-inoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse;

1.5.1.24 Spills and accidents which result in overt or potential exposures to organisms containing rDNA molecules are immediately reported to the laboratory director and to the IBC. Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained;

1.5.1.25 Baseline serum samples for all laboratory and other personnel at-risk should be collected and stored for reference purposes. Additional serum specimens may be collected periodically depending on the agents handled or the function of the laboratory;

1.5.1.26 A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures;

1.5.1.27 Alternative selection of containment equipment is possible. Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified can be conducted in the BL3 laboratory, using containment equipment specified for the BL2 level of physical containment. Experimental procedures involving a host-vector system that provides a one-step lower level of biological containment than that specified can be conducted in the BL3 laboratory using containment equipment specified for the BL4 level of physical containment. Alternative combination of containment safeguards.

1.5.2 Containment Equipment

Biological safety cabinets (Class I, II, or III) (See Part IV, Section 4) or other appropriate combinations of personal, protective or physical containment devices (e.g., special protective clothing, masks, gloves, respirators, centrifuge, safety cups, sealed centrifuge rotors, and containment caging for animals) are used for all activities with organisms containing rDNA molecules, which pose a threat of aerosol exposure. These include: manipulation of cultures and of clinical or environmental materials which may be a source of aerosols; the aerosol challenge of experimental animals, harvesting infected tissues or fluids from experimental animals and embryonate eggs, and necropsy of experimental animals;

1.5.3 Laboratory Facilities

1.5.3.1 The laboratory is separated from areas which are open to unrestricted traffic flow within the building. Passage through two sets of doors is the basic requirement for entry into the laboratory from access corridor or other contiguous areas. Physical separation of the high containment laboratory from access corridors or other laboratories or activities may also be provided by a double-doored clothes change room (showers may be included), air lock, or other access facility which requires passage through two sets of doors before entering the laboratory;

1.5.3.2 The interior surfaces of walls, floors, and ceilings are water resistant so that they can be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate decontamination of the area;

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

1.5.3.4 Laboratory furniture is sturdy and spaces between benches, cabinets, and

equipment are accessible for cleaning;

1.5.3.5 Each laboratory contains a sink for hand washing. The sink may be operated by foot, by elbow, or automatically and is located near the laboratory exit door;

1.5.3.6 Windows in the laboratory are closed and sealed;

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

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

1.5.3.9 A ducted exhaust air ventilation system is provided. This system creates directional airflow that draws air into the laboratory through the entry area. The exhaust air is not recirculated to any other area of the building, is discharged to the outside, and is dispersed away from the occupied areas and air intakes. Personnel must verify that the direction of the airflow (into the laboratory) is proper. The exhaust air from the laboratory room should be filtered before it is discharged to the outside to be sure it is not contaminated;

1.5.3.10 The HEPA-filtered exhaust air from Class I or Class II biological safety cabinets may be recirculated within the laboratory if the cabinet is tested and certified at least every 12 months. If the HEPA-filtered exhaust air from Class I or II biological safety cabinets is to be discharged to the outside through the building exhaust air system, it should be connected in a manner [e.g., thimble unit connection (12)] that avoids any interference with the air balance of the cabinets or building exhaust system.

1.6 Biosafety Level 4 (BL4)

BL4 provides the most stringent containment conditions. All requirements listed in BL3 are applicable to BL4. Standard microbiological practices should be followed.

1.6.1 Procedures

1.6.1.1 Work surfaces are decontaminated at least once a day and immediately after any spill of viable material;

1.6.1.2 Only mechanical pipetting devices are used;

1.6.1.3 Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the laboratory;

1.6.1.4 All procedures are performed carefully to minimize the creation of aerosols;

1.6.1.5 Biological materials to be removed from the Class III cabinets or from the maximum containment laboratory in a viable or intact state are transferred to a non-breakable, sealed primary container, and then enclosed in a non-breakable, sealed secondary container, which is removed from the facility through a disinfectant dunk tank, fumigation chamber, or an air lock designed for this purpose;

1.6.1.6 No material, except biological materials that are to remain in a viable or intact state, is removed from the maximum containment laboratory unless it has been autoclaved or decontaminated. Equipment or material that might be damaged by high temperatures, or steam is decontaminated by gaseous or vapor methods in an airlock or chamber designed for that purpose;

1.6.1.7 Only persons whose presence are required for program or support purposes in the facility or individual laboratory rooms are authorized to enter. The laboratory director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. Access to the facility is limited by means of secure, locked doors; accessibility is managed by the laboratory director, biohazards control officer, or other persons responsible for the physical security of the facility. Before entering, persons are advised of the potential biohazards and instructed on appropriate safeguards to ensure their safety. Authorized persons comply with the instructions and all other applicable entry and exit procedures. A logbook signed by all personnel indicates the date and time of each entry and exit. Practical and effective protocols for emergency situations are established;

1.6.1.8 Personnel enter and leave the facility only through the clothing change and

shower rooms. Personnel shower every time they leave the facility; personnel use the airlocks to enter or leave the laboratory only in an emergency;

1.6.1.9 Street clothing is removed in the outer clothing change room and kept there. Complete laboratory clothing, including undergarments, pants, and shirts or jumpsuits, shoes, and gloves, is provided and used by all personnel entering the facility. Head covers are provided for personnel who do not wash their hair during the exit shower. When leaving the laboratory and before proceeding into the shower area, personnel remove their laboratory clothing and store it in a locker or hamper in the inner change room;

1.6.1.10 When materials that have organisms containing rDNA molecules or experimental animals are present in the laboratory or animal rooms, a hazard warning sign incorporating the universal biohazard symbol (Appendix 5) is posted on all access doors. The sign identifies the agent, lists the name of the laboratory director or other responsible person(s), and indicates any special requirements for entering the area (e.g., the need for immunization or respirators); 1.6.1.11 Supplies and materials needed in the facility are brought in through the double-doored autoclave, fumigation chamber, or airlock which is appropriately decontaminated between each use. After securing the outer doors, personnel within the facility retrieve the materials by opening the interior doors or the autoclave, fumigation chamber, or airlock. These doors are secured after materials are brought into the facility. An insect and rodent control program is in effect as certified by a licensed pest control operator for all levels;

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

1.6.1.13 Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral part of unit) are used for the injection or aspiration of fluids containing organisms that contain rDNA molecules. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be placed in a puncture-resistant container and decontaminated, preferably by autoclaving before discard or reuse. Whenever possible, cannulas are used instead of sharp needles (e.g., gavage);

1.6.1.14 A system is set up for reporting laboratory accidents and exposures and employee absenteeism, and for the medical surveillance of potential laboratory-associated illnesses. Written records are prepared and maintained. An essential adjunct to such a reporting-surveillance system is the availability of a facility for quarantine, isolation, and medical care of personnel with potential or known laboratory-associated illnesses;

1.6.1.15 Laboratory animals involved in experiments requiring BL4 level physical containment shall be housed either in cages contained in Class III cabinets or in partial containment caging systems such as Horsfall units (11), open cages placed in ventilated enclosures, or solid-wall and bottom cages placed on holding racks equipped with ultraviolet irradiation lamps and reflectors that are located in a specially designed area in which all personnel are required to wear one-piece positive pressure suits;

1.6.1.16 Alternative selection of containment equipment is possible. Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified can be conducted in the BL4 facility using containment equipment requirements specified for the BL3 level of physical containment. Alternative combinations of containment safeguards.

1.6.2 Containment Equipment

All procedures within the facility with agents assigned to BL4 are conducted in the Class III biological safety cabinet; or in Class I or II biological safety cabinets used in conjunction with one-piece positive pressure personnel suits ventilated by a life-support system.

1.6.3 Laboratory Facilities

1.6.3.1 The maximum containment facility consists of either a separate building or a clearly demarcated and isolated zone within a building. Outer and inner change rooms separated by a shower are provided for personnel entering and leaving the facility. A double-doored autoclave, fumigation chamber, or ventilated air lock is provided for passage of materials, supplies, or equipment that are not brought into the facility through the change room;

1.6.3.2 Walls, floors, and ceilings of the facility are constructed to form a sealed internal shell that facilitates fumigation and is animal and insect-proof. The internal surfaces of this shell are resistant to liquids and chemicals, thus facilitating cleaning and decontamination of the area. All penetrations in these structures and surfaces are sealed. Any drains in the floors contain traps filled with a chemical disinfectant of demonstrated efficacy against the target agent, and these are connected directly to the liquid waste decontamination system. Sewer and other ventilation lines contain HEPA filters;

1.6.3.3 Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes are arranged to minimize the horizontal surface area on which dust can settle;

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

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

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

1.6.3.7 If there is a central vacuum system, it does not serve areas outside the facility. In-line HEPA filters are placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement. Other liquid and gas services to the facility are protected by devices that prevent backflow;

1.6.3.8 If water fountains are provided, they are foot-operated and are located in the facility corridors outside the laboratory. The water service to the fountain is not connected to the back-flow-protected distribution system supplying water to the laboratory areas;

1.6.3.9 Access doors to the laboratory are self-closing and can be locked. All windows are breakage resistant; 1.6.3.10 A double-doored autoclave is provided for decontaminating materials passing out of the facility. The autoclave door, which opens to the area external to the facility, is sealed to the outer wall and automatically controlled so that the outside door can be opened only after the autoclave "sterilization" cycle has been completed;

1.6.3.11 A pass-through dunk tank, fumigation chamber, or an equivalent decontamination method is provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the facility;

1.6.3.12 Liquid effluents from laboratory sinks, biological safety cabinets, floors, and autoclave chambers are decontaminated by heat treatment before being released from the maximum containment facility. Liquid wastes from shower rooms and toilets may be decontaminated with chemical disinfectants or by heat in the liquid waste decontamination system. The procedure used for heat decontamination of liquid wastes is evaluated mechanically and biologically by using a recording thermometer and an indicator microorganism with a defined heat susceptibility pattern. If liquid wastes from the shower room are decontaminated with chemical disinfectants, the chemical used should be of demonstrated efficacy against the target or indicator microorganisms;

1.6.3.13 An individual supply and exhaust air ventilation system is provided. The system maintains pressure differentials and directional airflow as required to assure flows inward from areas outside of the facility toward areas of highest potential risk

within the facility. Manometers are used to sense pressure differentials between adjacent areas maintained at different pressure levels. If a system malfunctions, the manometers sound an alarm. The supply and exhaust airflow is interlocked to assure inward (or zero) airflow at all times;

1.6.3.14 The exhaust air from the facility is filtered through HEPA filters and discharged to the outside so that it is dispersed away from occupied buildings and air intakes. Within the facility, the filters are located as near the laboratories as practicable in order to reduce the length of potentially contaminated air ducts. The filter chambers are designed to allow in situ decontamination before filters are removed and to facilitate certification testing after they are replaced. Coarse filters and HEPA filters are provided to treat air supplied to the facility in order to increase the lifetime of the exhaust HEPA filters and to protect the air supply system should air pressures become unbalanced in the laboratory;

1.6.3.15 The treated exhaust air from Class I and II biological safety cabinets can be discharged into the laboratory room environment or outside through the facility air exhaust system. If exhaust air from Class I or II biological safety cabinets is discharged into the laboratory, the cabinets are tested and certified at 6 month intervals. The exhaust air from Class III biological safety cabinets is discharged, without recirculation through two sets of HEPA filters in series, via the facility exhaust air system. If the treated exhaust air from any of these cabinets is discharged to the outside through the facility exhaust air system, the treated exhaust air is connected to this system in a manner [e.g., thimble unit connection (12)] that avoids any interference with the air balance of the cabinets or the facility exhaust air system;

1.6.3.16 A specially designed suit area may be provided in the facility. Personnel who enter this area wear a one-piece positive pressure suit that is ventilated by a life-support system. The life-support system includes alarms and emergency backup breathing air tanks. Entry to this area is through an airlock fitted with airtight doors. A chemical shower is provided to decontaminate the surface of the suit before the worker leaves the area. The exhaust air from the suit area is filtered by two sets of HEPA filters installed in series. A duplicate filtration unit, exhaust fan, and an automatically starting emergency power source are provided. The air pressure within the suit area is lower than that in any adjacent area. Emergency lighting and communication systems are provided. All penetrations into the internal shell of the suit area are sealed. A double-doored autoclave is provided for decontaminating waste materials to be removed from the suit area.

2. *Biological Containment*

2.1 In considering biological containment, the vector (plasmid, organelle, or virus) for the rDNA and the host (bacterial, plant or animal cell) in which the vector is propagated in the laboratory will be considered together. In any combination of vector and host, the biological containment must be chosen or constructed so that the following types of "escape" are minimized: (i) survival of the vector in its host outside the laboratory, and (ii) transmission of the vector from the propagation host to other non-laboratory hosts.

The following levels of biological containment for host-vector systems (HV) for prokaryotes will be established; and specific criteria will depend on the organisms to be used:

2.1.1 **HV1.** A host-vector system that requires a moderate level of containment. Specific systems follow :

EK1: The host is always *E. coli* K-12 or a derivative thereof, and the vectors include non-conjugative plasmids (e.g., PSC101, Co(F) or derivatives thereof (1-7), and variants of bacteriophage such as lambda (8-15). The *E. coli* K-12 hosts should not contain configuration proficient plasmids, whether autonomous or integrated or generalized transducing phages.

Other HV1. Hosts and vectors shall be, at a minimum, comparable in containment to *E. coli* K-12 with a non-conjugative plasmid or bacteriophage vector. The data to be considered and a mechanism for approval of such HV1 systems are described in Section 2.2.

2.1.2 **HV2.** These are host vector systems shown to provide a high level of biological

containment as demonstrated by data from suitable tests performed in the laboratory. Escape of the rDNA either via survival of the organisms or via transmission of rDNA to other organisms should be less 1/104 under specified conditions. Specific systems are as follows:

EK2: For EK2 host vector systems in which the vector is a plasmid, no more than in 104 host cells should be able to perpetuate a cloned DNA fragment under the specified non-permissive laboratory conditions designed to represent the natural environment, either by survival of the original host or as a consequence of transmission of the cloned DNA fragment.

For EK2 host vector system in which the vector is a phage, no more than one in 104 phage particles should be able to perpetuate a cloned DNA fragment under the specified non-permissive laboratory conditions designed to represent the natural environment either (i) as a prophage (in the inserted or plasmid form) in the laboratory host used for phage propagation or (ii) by surviving in natural environments and transferring a cloned DNA fragment to other hosts (or their resident prophages).

2.2 Safety level as DNA donors of primitive eukaryotes and prokaryotes.

2.2.1 Organisms used as DNA donor that require P4-B1 containments :

Bartonella

B. bacilliformis

Clostridium C. botulinum

C. tetani

Corynebacterium

C. diphtheriae

Mycoplasma

M. mycoides

Pasteurella

P. multocida

(B:6, E:6, A:5, A:8, A:9)

Pseudomonas P. mallei

(Actinobacillus mallei)

P. pseudomallei

Shigella S. dysenteriae

Yersinia Y. pestis

(Y. pseudotuberculosis subsp. pestis)

2.2.2 Organisms used as donor that require P3-B1 or P2-B2 containments :

Bacillus

B. abortus

Brucella

B. abortus

B. melitensis

B. suis

Coccidioides

C. immitis

Cryptococcus

C. neoformans

Francisella

F. tularensis

Histoplasma

H. capsulatum

H. duboisii

Mycobacterium

M. africanum
M. bovis
M. tuberculosis

Salmonella
S. paratyphi-A
S.typhi

2.2.3 Organisms used as DNA donor that require P2-B1 or P2-B2 containments :

Actinobacillus
A. mallei
(Pseudomonas mallei)

Actinomyces
A. bovis
A. israelii
A. naeslundii

Aeromonas
A. hydrophila.rm67
(Toxin producing strains)
A. punctata
(Toxin producing strains)

Arizona
A. hinshawii
(all antigenic type)

Bacillus
B. cereus (Toxin producing strains)

Blastomyces
B. dermatitidis

Bordetella
All spp.

Borrelia
All spp.

Brucella
B. canis

Calymmatobacterium
C. granulomatis

Campylobacter
All spp.

Clostridium
C. chauvoei
C. difficile
C. haemolyticum
C. histolyticum
C. novyi
C. perfringens
(Toxin producing strain)
C. septicum

Corynebacterium
C. equi
C. haemolyticum
C. pseudotuberculosis
C. pyogenis
C. renale

Entamoeba
E. histolytica

Erysipelothrix
E. rhusiopathiae
E. insidiosus

Escherichia
E. coli (all antigenic types with pathogenicity to intestine)

Haemophilus
H. ducreyi
H. influenzae

Hartmannella
All spp.

Herellea
H. vaginicola

Klebsiella
All spp.

Legionella
L. pneumophila

Leishmania
All spp.

Leptospira
L. interrogans
(all antigenic types)

Listeria L. monocytogenes

Moraxella
M. polymorpha

Moraxella
All spp.

Mycobacterium
M. avium
M. intracellulare complex
M. kansasii
M. marinum
M. paratuberculosis
M. avium-intracellulare complex
M. ulcerans

Mycoplasma
M. pneumoniae

Naegleria
All spp.

Neisseria
N. gonorrhoeae
N. meningitidis

Nocardia
N. asteroides
N. brasiliensis
N. caviae
N. farcinica

Paracoccidiosis

P. brasiliensis

Pasteurella
All spp. except
P. multocida

Plasmodium
P. falciparum
P. malariae
P. ovale
P. vivax.rm67
Simian malarial parasites

Plesiomonas
P. shigelloides

Salmonella
All serotypes except
S. paratyphi-A and
S. typhi
S. dysenteriae

Sphaerophorus
S. necrophorus

Staphylococcus
S. aureus

Streptococcus
S. pneumoniae
S. pyogenes

Treponema
T. carateum
T. pallidum
T. pertenue

Trichinella
T. spiralis

Toxocara
T. canis

Toxoplasma *T. gondii*

Trypanosoma
T. cruzi
T. gambiense
T. rhodesiense

Vibro
v. cholera
(including Biotype El Tor)

Yersinia
Y. enterocolitica
Y. pseudotuberculosis
(except *Y. pestis*)
(*Y. pseudotuberculosis* subsp. *pestis*)

2.3 Safety level for DNA donor of virus, rickettsia, and Chlamydia of Protokaryota excluding primitive organisms:

2.3.1 Organisms used as DNA donor that require P3-B1 or P2-B2 containments:

California encephalitis virus
Chikungunia virus
Chlamydia psittaci

Herpes virus ateles
Herpes virus saimiri
HIV
Hog cholera virus
HTLV - ATLV
HTLV - I
Japanese encephalitis virus
La Crosse virus
LCM virus
Monkeypox virus
Murray Valley encephalitis virus
O'nyong-nyong virus
Powassan virus
Rabies street virus
St. Louis encephalitis virus
Tacaribe virus
Vesicular stomatitis virus
West Nile virus

2.3.2 Organisms used as DNA donor that require

P2-B1 or P1-B2 containments:
Avian reticuloendotheliosis virus
Batai virus
BK virus
Bovine papilloma virus
Chlamydia trachmatics
Cowpox virus
Coxsackie virus (A, B)
Cytomegalovirus (human, animal)
Dengue virus (1-4)
Eastern equine encephalitis virus
EB virus
Echovirus (1.-34)
Ectromelia virus
Enterovirus (68-71)
Equine infectious anemia virus
Equine rhinopneumonitis virus
Hepatitis A virus
Hepatitis B virus
Hepatitis non A non B virus
Herpo simplex virus (1, 2)
Human adenovirus
Human influenza virus (A, B, C)
Human wart virus (Human papilloma virus)
HVJ
JC virus
Mammalian retrovirus (except HIV, HTLV-I,
(ATLV) and HTLV-II)
Measles virus Molluscum contagiosum virus
Mouse hepatitis virus
Mumps virus
NDV
Parainfluenza virus (1-4)
Pichinde virus
Poliovirus (1-3)
Polyoma virus
Pseudorabies virus
Rabies (fixed, attenuated) virus
Rhinovirus
Rinderpest virus (vaccine strain)

Rotavirus
Rubella virus
Semliki Forest virus
SSPE agent
SV 40
Tanapox virus
Vaccinia virus
Varicella virus
Western equine encephalitis virus

2.3.3 Organisms used as DNA donor that require

P1-B1 or P1-B2 containments:

Aino virus
Akabane virus
Avian adenovirus
Avian encephalomyelitis virus
Avian enterovirus
Avian influenza virus
Avian poxvirus
Avian retrovirus (except Avian reticuloendotheliosis virus)
Bluetongue virus
Bovine adenovirus
Bovine enterovirus
Bunyamwera virus
Canine distemper virus
Coronavirus
Duck hepatitis virus
Equine influenza virus
Getah virus
Langat virus
Live virus vaccine strains (except Rinderpest vaccine strain)
Lucke virus
Marek's disease virus
Parvovirus
Poikilothermal vertebrate retrovirus
Porcine adenovirus
Reovirus (1-3)
Ross River virus
Shope fibroma virus
Simbu virus
Sindbis virus
Swine influenza virus
Swinepox virus
Viroids
Fish viruses (Limit to IPN, IHN, EVA, EVE, LV)
Insect viruses (except insect viruses such as arbovirus, which are pathogenic vertebrate)
Plant viruses

2.4 Host-vector systems of which a high level of safety (requires PI level containments) has been confirmed when primitive eukaryote or prokaryote not listed in 2.2.1, 2.2.2, and 2.2.3 and their viruses are used as a DNA donor:

AA (a host-vector system with *Azotobacter aceti* as a host and plasmid or bacteriophage as a vector)

BA (a host-vector system with *Bacillus amyloliquefaciens* as a host and plasmid or bacteriophage as a vector)

BB (a host-vector system with *Bacillus brevis* as a host and plasmid or bacteriophage as a vector)

BF (a host-vector system with *Brevibacterium flavum* as a host and plasmid or bacteriophage

as a vector)

BL (a host-vector system with *Brevibacterium lactofermentum* as a host and plasmid or bacteriophage as a vector)

BSt (a host-vector system with *Bacillus stearothermophilus* as a host and plasmid or bacteriophage as a vector)

CH (a host-vector system with *Corynebacterium herculis* as a host and plasmid or bacteriophage as a vector)

PP (a host-vector system with *Pseudomonas putida* as a host and plasmid or bacteriophage as a vector)

SK (a host-vector system with *Streptomyces kasugaensis* as a host and plasmid or bacteriophage as a vector)

SL (a host-vector system with *Streptomyces lividans* as a host and plasmid or bacteriophage as a vector)

SP (a host-vector system with *Schizosaccharomyces pombe* as a host and plasmid or bacteriophage as a vector)

ZR (a host-vector system with *Zygosaccharomyces rouxii* as a host and plasmid or bacteriophage as a vector)

2.5 Certification of Host-Vector Systems

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

The Chairperson
National Committee on Biosafety of the Philippines
Department of Science and Technology
Bicutan, Taguig, Metro Manila

2.5.2 Host vector systems that are proposed for certification will be reviewed by the NCBP. Prior to this, a review of the data on construction, properties, and testing of the proposed host-vector system will be made by a working group composed of one or more members of the NCBP and other persons chosen because of their expertise in evaluating such data. The NCBP will then evaluate the report of the working group and any other available information at a regular meeting.

The Chairperson of the NCBP is responsible for certification after receiving the advice of the working group. Minor modifications of existing certified host-vector systems, i.e., those of minimal or no consequence to the properties relevant to containment, may be certified by the NCBP Chairperson.

2.5.3 When a new host-vector system is certified, NCBP sends a notice of the certification to the applicant and to all IBCs and publishes it. Copies of a list of all currently certified host-vector systems may be obtained from NCBP at any time.

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

2.5.5 Certification of a given system does not extend to modifications of either the host or vector component of that system. Such modified systems must be independently certified by the NCBP Chairperson. If modifications are minor, it may only be necessary for the investigator to submit data showing that the modifications have either improved or not impaired the major phenotypic traits on which the containment of the system depends. Substantial modifications of a certified system require the submission of complete testing data.

2.6 Data to be Submitted for Certification

2.6.1 HV1 systems other than E. coli K-12. The following types of data shall be submitted, modified as appropriate for the particular system being considered: (i) a description of the organism and vector, the strain's natural habitat and growth requirements; its physiological properties, particularly those related to its reproduction and survival and the mechanisms by which it exchanges genetic information; the range of organisms with which this organism normally exchanges genetic information and what sort of information is exchanged; and any relevant information on its pathogenicity or toxicity; (ii) a description of the history of the particular strains and vectors to be used, including data on any mutations that render this organism less able to survive or transmit genetic information; and (iii) a general description of the range of experiments contemplated with emphasis on the need for developing such an HV1 system.

2.6.2 HV2 Systems. Investigators planning to request HV2 certification for host-vector systems can obtain instructions from NCBP concerning data to be submitted. In general, the following types of data are required: (i) description of construction steps with indication of source, properties, and manner of introduction of genetic traits; (ii) quantitative data on the stability of genetic traits that contribute to the containment of the system; (iii) data on the survival of the host-vector system under non-permissive laboratory conditions designed to represent the relevant natural environment; (iv) data on transmissibility of the vector and/or a clone DNA fragment under both permissive and non-permissive conditions; (v) data on all other properties of the system which affect containment and utility, including information on yields of phage or plasmid molecules, ease of DNA isolation, and ease of transfection or transformation. In some cases, the investigator may be asked to submit data on survival and vector transmissibility from experiments in which the host vector is fed to laboratory animals and human subjects. Such in vivo data may be required to confirm the validity of predicting in vivo survival on the basis of in vitro experiments. Data must be submitted in writing to NCBP. A period of 10 to 12 weeks is normally required for review and circulation of the data. Investigators are encouraged to publish their data on the construction, properties, and testing of proposed HV2 systems before the system is considered by the NCBP and its sub-committee.

3. Physical Containment for Large Scale Uses of Organisms Containing Recombinant DNA Molecules

This part of the Guidelines specifies physical containment guidelines for large scale (greater than 10 liters of culture) research or production involving viable organisms containing rDNA molecules. It shall apply to large scale research or production activities.

All provisions of the Guidelines shall apply to large scale research or production activities, with the following modifications:

- The institution shall appoint Biological Safety Officer(s) (BSO) if it engages in large scale research or production activities involving viable organisms containing rDNA molecules.
- The institution shall establish and maintain a health surveillance program for personnel engaged in large scale research or production activities involving viable organisms containing rDNA molecules, which require BL3 containment at the laboratory scale. The program shall include pre assignment and periodic physical and medical examinations; collection, maintenance, and analysis of serum specimens for monitoring serologic changes that may result from the employee's work experience; and provisions for investigating any serious, unusual, or extended illnesses of employees to determine possible occupational origin.

3.1 Selecting Physical Containment Levels

The selection of the physical containment level required for rDNA research or production involving more than 10 liters of culture is based on the containment guidelines established in Section 5 of Part IV. For large scale research or production, three physical containment levels are established: BL1-LS, BL2-LS, and BL3-LS.

The BL1-LS level of physical containment level required for large-scale research or production of viable organisms containing rDNA molecules that require BL1 containment at the laboratory scale. The BL2-LS level is required for large scale research or production of viable organisms containing rDNA molecules that require BL2 containment at the laboratory scale.

The BL3-LS level is required for large scale research or production of viable organisms containing rDNA molecules the require BL3 containment at the laboratory scale.

No provisions are made for large scale research or production of viable organisms containing

rDNA molecules that require BL4 containment at the laboratory scale. If necessary, these requirements will be established by NCBP on an individual basis.

3.1.1 BL1-LS Level

3.1.1.1 Cultures of viable organisms containing rDNA molecules shall be handled in a closed system (e.g., closed vessel used for propagating and growing cultures) or other primary containment equipment (e.g., biological safety cabinet containing a centrifuge used to process culture fluids) designed to reduce the potential for escape of viable organisms. Volumes less than 10 liters may be handled outside of a closed system or other primary containment equipment, provided all physical containment requirements specified in Part IV, Section 1.3 are met.

3.1.1.2 Culture fluids (except as allowed in 3.1.1.3) shall not be removed from devised system or other primary containment equipment unless the viable organisms containing rDNA molecules have been inactivated by a validation inactivation procedure. A validation inactivation procedure is one that has been demonstrated to be effective using the organism that will serve as the host for propagating the rDNA molecules.

3.1.1.3 Sample collection from a closed system and transferring culture fluids from closed system to another shall be done in a manner which minimizes the release of aerosols or contamination of exposed surfaces.

3.1.1.4 Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters that have efficiencies equivalent to HEPA filters or by other equivalent procedures (e.g., incineration) to minimize the release of viable organisms containing rDNA molecules.

3.1.1.5 A closed system or other primary containment that has held viable organisms containing rDNA molecules shall not be opened for maintenance or other purposes unless it has been sterilized by a validated sterilization procedure. A validated sterilization procedure is one that has been demonstrated to be effective using the organism that will serve as the host for propagating the rDNA molecules.

3.1.1.6 Emergency plans required to cover accidental spills and personnel contamination shall include methods and procedures for handling large losses of culture on an emergency basis.

3.1.2 BL2-LS Level

3.1.2.1 Cultures of viable organisms containing rDNA molecules shall be handled in a closed system (e.g., closed vessel used for propagating and growing cultures) or other primary containment equipment (e.g., Class III biological safety cabinet containing a centrifuge used to process culture fluids) designed to prevent the escape of viable organisms. Volumes less than ten (10) liters may be handled outside of a closed system or other primary containment equipment, provided all physical containment requirements specified in Part IV, Section 1.4 are met.

3.1.2.2 Culture fluids (except as allowed in Section 3.1.1.3) shall not be removed from a closed system or other primary containment equipment unless the viable organisms containing rDNA molecules have been inactivated by a validated inactivation procedure. A validated inactivation procedure is one that has been demonstrated to be effective using the organisms that will serve as the host for propagating the rDNA molecules.

3.1.2.3 Sample collection from a closed system, the addition of materials to a closed system, and the transfer of culture fluids from one closed system to another shall be done in a manner that prevents the release of aerosols or contamination of exposed surfaces.

3.1.2.4 Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters that have efficiencies to HEPA filters or by other equivalent procedures (e.g., incineration) to prevent the release of viable organisms containing rDNA molecules to the environment.

3.1.2.5 A close system or other primary containment equipment that has held viable organisms containing rDNA molecules shall not be opened for maintenance or other purposes unless it has been sterilized by a validated sterilization procedure. A validated sterilization procedure is one that has been demonstrated to be effective using the organisms that will serve as the host for propagating the rDNA molecules.

3.1.2.6 Rotating seals and other mechanical devices directly associated with a closed system used for propagating and growing viable organisms containing rDNA molecules shall be designed to prevent leakage or shall be fully enclosed in ventilated housings that are exhausted through filters that have efficiencies equivalent to HEPA filters or through other equivalent treatment devices.

3.1.2.7 A closed system/containment equipment used for propagating and growing viable organisms containing rDNA molecules shall include monitoring or sensing devices that monitor the integrity of containment during operations.

3.1.2.8 A closed system used for propagating and growing viable organisms containing the rDNA molecules shall be tested for integrity of the containment features using the organism that will serve as the host for propagating rDNA molecules. Testing shall be conducted before viable organisms containing rDNA molecules are introduced and after essential containment features have been modified or replaced. Procedures and methods used in the testing shall be appropriate for the equipment design and for recovery and demonstration of the test organism. Records of tests and results shall be maintained on file.

3.1.2.9 A closed system used for propagating and growing viable organisms containing rDNA molecules shall be permanently identified. This identification shall be used in all records reflecting testing, operation, and maintenance, and in all documentation relating to use of this equipment for research or production activities involving viable organisms containing rDNA molecules.

3.1.2.10 The universal biohazard sign (Appendix 5) shall be posted on each closed system and primary containment equipment when used to contain viable organisms containing rDNA molecules.

3.1.2.11 Emergency plans required to cover accidental spills and personnel contamination shall include methods and procedures for handling large losses of culture on an emergency basis.

3.1.3 BL3-LS Level

3.1.3.1 Cultures of viable organisms containing rDNA molecules shall be handled in a closed system (e.g., closed vessels used for propagating and growing cultures) or other primary containment equipment (e.g., Class III biological safety cabinet containing a centrifuge used to process culture fluids) which is designed to prevent the escape of viable organisms. Volumes less than 10 liters may be handled outside of a closed system, provided all physical containment requirements specified in Section 1.5 of Part IV are met.

3.1.3.2 Culture fluids (except as allowed in Section 3.1.1.3) shall not be removed from a closed system or other primary containment equipment unless the viable organisms containing rDNA molecules have been inactivated by a validated inactivation procedure. A validated inactivation procedure is one which has been demonstrated to be effective using the organisms that will serve as the host for propagating the rDNA molecules.

3.1.3.3 Sample collection from a closed system, the addition of materials to a closed system, and the transfer of culture fluids from one closed system to another shall be done in a manner which prevents the release of aerosols or contamination of exposed surfaces.

3.1.3.4 Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters which have efficiencies equivalent to HEPA filters or by other equivalent procedures (e.g., incineration) to prevent the release of viable organisms containing rDNA molecules to the environment.

3.1.3.5 A closed system or other primary containment equipment that has held viable organisms containing rDNA molecules shall not be opened for maintenance or other purposes unless it has been sterilized by a validated sterilization procedure. A validated sterilization procedure is one that has been demonstrated to be effective using the organisms that will serve as the host for propagating the rDNA molecules.

3.1.3.6 A closed system used for propagating and growing viable organisms containing rDNA molecules shall be operated so that the space above the culture level will be maintained at a pressure as low as possible, consistent with equipment design, to maintain the integrity of containment features.

3.1.3.7 Rotating seals and other mechanical devices directly associated with a closed system used to contain viable organisms containing rDNA molecules shall be designed to prevent leakage or shall be fully enclosed in ventilated housings that are exhausted through filters that have efficiencies equivalent to HEPA filters or through other equivalent treatment devices.

3.1.3.8 A closed system used for propagating and growing viable organisms containing rDNA molecules, and other primary containment equipment used to contain operations involving viable organisms containing rDNA molecules shall include monitoring or sensing devices that monitor the integrity of containment during operations.

3.1.3.9 A closed system used for propagating and growing viable organisms containing rDNA molecules shall be tested for integrity of the containment features using the organisms that will serve as the host for propagating the rDNA molecules. Testing shall be conducted before viable organisms containing rDNA molecules are introduced and after essential containment features have been modified or replaced. Procedures and methods used in the testing shall be appropriate for the equipment design and for recovery and demonstration of the test organism. Records of tests and results shall be maintained on file.

3.1.3.10 A closed system used for propagating and growing of viable organisms containing rDNA molecules shall be permanently identified. This identification shall be used in all records reflecting testing, operation, and maintenance and in all documentation relating to the use of this equipment for research production activities involving viable organisms containing rDNA molecules.

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

3.1.3.12 Emergency plans required to cover accidental spills and personnel contamination shall include methods and procedures for handling large losses of culture on an emergency basis.

3.1.3.13 Closed systems and other primary containment equipment used in handling cultures of viable organisms containing rDNA molecules shall be located within a controlled area that meets the following requirements:

- a. The controlled area shall have a separate entry area. The entry area shall be a double doored space such as an air lock, anteroom, or change room that separates the controlled area from the balance of the facility.
- b. The surfaces of walls, ceilings, and floors in the controlled area shall be such that they can be readily cleaned and decontaminated.
- c. Penetrations into the controlled area shall be sealed to permit liquid or vapor phase space decontamination.
- d. All utilities and service or process piping and wiring entering the controlled area shall be protected against contamination.
- e. Hand-washing facilities equipped with valves that can be operated by foot, by elbow, or automatically shall be located at each major work area and near

each primary exit.

f. A shower facility shall be provided. This facility shall be located near the controlled area.

g. The controlled area shall be designed to preclude release of culture fluids outside the controlled area when an accidental spill or release from the closed systems or other primary containment equipment occurs.

h. The controlled area shall have a ventilation system that is capable of controlling air movement. The movement of air shall be from areas of lower contamination potential to areas of higher contamination potential. If the ventilation system provides positive pressure air supply, the system shall operate in a manner that prevents the reversal of the direction of air movement or shall be equipped with an alarm that would be activated when reversal in the direction of air movement occurs. The exhaust air from the controlled area shall not be recirculated to other areas of the facility. The exhaust air from the controlled area may be discharged to the outdoors without filtration or other means of effectively reducing an accidental aerosol burden, provided that it can be dispersed clear of occupied buildings and air intakes.

3.1.3.14 The following personnel and operational practices shall be required:

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

b. Persons entering the controlled area shall exchange or cover their personal clothing with work garments such as jumpsuits, laboratory coats, pants and shirts, head cover, and shoes or shoe covers. On exit from the controlled area, the work clothing may be stored in a locker separate from that used for personal clothing or discarded for laundering. Clothing shall be decontaminated before laundering.

c. Entry into the controlled area when work is in progress shall be restricted to those persons required to meet program or support needs. Prior to entry, all persons shall be informed of the operating practices, emergency procedures, and the nature of the work conducted.

d. Access doors to the controlled area shall be kept closed, except as necessary for access, while work is in progress. Service doors leading directly outdoors shall be sealed and locked while work is in progress.

e. Persons under 18 years of age shall not be permitted to enter the controlled area.

f. The universal biohazard sign shall be posted on entry doors to the controlled area and all internal doors when any work involving the organism is in progress. This includes periods when decontamination procedures are in progress. The sign posted on the entry doors to the controlled area shall include a statement of agents in use and personnel authorized to enter the controlled area.

g. Persons shall wash their hands when leaving the controlled area.

h. The controlled area shall be kept neat and clean.

i. Eating, drinking, smoking, and storage of food are prohibited in the controlled area.

j. An effective insect and rodent control program shall be maintained.

k. Persons working in the controlled area shall be trained in emergency procedures.

l. Equipment and materials required for the management of accidents involving viable organisms containing rDNA molecules shall be available in the controlled area.

m. The controlled area shall be decontaminated in accordance with established procedures following spills or other accidental release of viable organisms containing rDNA molecules.

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

4.1 **A Class I cabinet** is a ventilated cabinet for personnel protection; air in it flows inward, away from the operator. The exhaust air from this cabinet filters through a HEPA filter. This cabinet is used in three operational models: (1) with full-width open front, (2) with an installed front closures panel (having four 5 inch diameter openings) without gloves, and (3) with an installed front closure panel equipped with arm-length rubber gloves. The face velocity of the inward flow of air through the full width open front is 75 feet per minute or greater.

4.2 **A Class II cabinet** is a ventilated cabinet for personnel and product protection; it has an open front with inward airflow for personnel protection, and HEPA filtered mass recirculated airflow for product protection. The cabinet exhaust air is filtered through a HEPA filter. The face velocity of the inward flow of air through the full width open front is 75 feet per minute or greater.

4.3 **A Class III cabinet** is a closed front ventilated cabinet of gas-tight construction, which provides the highest level of personnel protection among biohazard safety cabinets. The interior of the cabinet is protected from contaminants outside of the cabinet. The cabinet is fitted with arm length rubber gloves and is operated under a negative pressure of at least 0.5 inch water gauge. All air supply is filtered through HEPA filters. Exhaust air is filtered through two HEPA filters or one HEPA filter and incinerator before being discharged to the outside environment.

5. Container Requirements

5.1 **Plants and plant parts.** All plants or plant parts, except seeds, cells, and sub cellular elements shall be packed in a sealed plastic bag of at least 5 mil thickness, inside a sturdy, sealed, leakproof, outer shipping container made of corrugated fiberboard, corrugated cardboard, or other material of equivalent strength.

5.2. **Seeds.** All seeds shall be transported in a sealed plastic bag of at least 5 mil thickness, inside a sealed metal container, which shall be placed inside a second sealed metal container. Shock absorbing cushioning material shall be placed between the inner and outer metal containers. Each metal container shall be independently capable of protecting the seeds and preventing spillage or escape. Each set of metal containers shall then be enclosed in a sturdy outer shipping container made of corrugated fiberboard, corrugated cardboard, wood or other material of equivalent strength.

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

5.3.1 Volume not exceeding 50 ml

Regulated materials not exceeding 50 ml shall be placed in a securely closed watertight container, primary container (test tube, vial, etc.) which shall be enclosed in a second, durable watertight container (secondary container). Several primary containers may be enclosed in a single secondary container, if the total volume of all the primary containers so enclosed does not exceed 50 ml. The space at the top, bottom, and sides between the primary and secondary containers shall contain sufficient nonparticulate absorbent material (e.g., paper towel) to absorb the entire contents of the primary container(s) in case of breakage or leakage. Each set of primary and secondary containers shall then be enclosed in an outer shipping container made of corrugated fiberboard, corrugated cardboard, wood, or other material of equivalent strength.

5.3.2 Volume exceeding 50 ml

Regulated materials that exceed a volume of 50 ml shall comply with requirements enumerated in Section 5.3.1. In addition, a shock absorbing material, in volume at least equal to that of the absorbent material between the primary and secondary containers, shall be placed at the top, bottom, and sides between the secondary container and the outer shipping container. Single primary containers shall not contain more than 1,000 ml of material. However, two or more primary containers whose combined volumes do not exceed 1,000 ml may be placed in a single, secondary container. The maximum amount of

microorganisms of etiologic agents, cells, or sub cellular elements which may be enclosed within a single outer shipping container shall not exceed 4,000 ml.

5.4 Insects, Mites, and Related Organisms

Insects (any life stage) shall be placed in an escape proof primary shipping container (insulated vacuum container, glass, metal, plastic, etc.) and sealed to prevent escape. Such primary container shall be placed securely within a secondary shipping container of crushproof styrofoam or other material of equivalent strength. One or more rigid ice packs may also be placed within the secondary shipping container, and sufficient packing material shall be added around the primary container to prevent movement of the primary shipping container. The secondary (styrofoam or other) container shall be placed securely within an outer shipping container made of corrugated fiberboard, corrugated cardboard, wood, or other material of equivalent strength.

5.5 Other Macroscopic Organisms

All macroscopic organisms that are not plants and which requires continuous access to atmospheric oxygen shall be placed in primary shipping made containers made of a sturdy, crushproof frame of wood, metal, or material of equivalent strength, surrounded by escaped proof mesh or netting of a strength and mesh size sufficient to prevent the escape of the smallest organism in the shipment, with edges and seams of the mesh or netting sealed to prevent escape organisms. Each primary shipping container shall be securely placed within a larger secondary shipping container made of wood, metal, or equivalent strength material. The primary and secondary shipping containers shall then be placed securely within an outer shipping container made of corrugated cardboard, wood, or other material of equivalent strength. The outer container may have air holes or spaces in the sides and/or ends of the container, provided that the outer shipping container must retain sufficient strength to prevent crushing of the primary and secondary shipping containers.

5.6 Illustration of suitable packaging and labeling of regulated materials.

