GUIDELINES FOR THE USE AND SAFETY OF GENETIC ENGINEERING TECHNIQUES OR RECOMBINANT DNA TECHNOLOGY

Inter-American Institute for Cooperation on Agriculture

Pan American Health Organization/World Health Organization

Organization of American States

International Office of Epizootics

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DEDICATION

Dr. Pedro N. Acha was responsible for coordinating the preparation and publication of these guidelines and for organizing the work of the Study Group. Without his enthusiasm and dedication, this difficult undertaking would not have been possible. He brought to the task many years of experience and his considerable expertise in the field.

Not that these challenges were new to Dr. Acha. As so often in the past, his energy and dynamism carried even the most difficult and complex projects to a successful conclusion.

We dedicate this work to the memory of Pedro N. Acha, an eminent professional and a dear friend, as testimony to his untiring efforts to improve the technology of agriculture and public health in the countries of the Americas.

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PROLOGUE

Cooperating multilateral technical agencies are facing the pressing need to coordinate activities in their various spheres of influence, given the very multidisciplinary nature and the similar focus of much of their work. This situation, together with the constraints imposed by the large external debt, which has proved so crippling for countries in the developing world, makes it all the more important for these agencies to seek combined, coordinated approaches that will permit them to obtain maximum return on the investments they are making.

Financial restrictions are not the only consideration. These agencies have acquired an expertise and knowledge that will continue to be required in the development and implementation of useful and effective programs. In fact, the sum total of the individual agencies efforts falls far short of what their potential effect could be if those efforts were coordinated among the various parties.

Few fields of contemporary science and technology hold forth more possibilities and greater expectations than biotechnology. That potential alone is more than enough to justify that the term "biotechnology" appear in the future planning and strategies of an ever-growing number of agencies and institutions. Similarly, it justifies that, in undertaking biotechnological activities, agencies and institutions orchestrate their work and, together, seek to achieve a maximum effect. Moreover, in this area--as in any other--the concern for adopting common regulatory measures is a primary reason for the concerted efforts of interested parties and agencies.

The agencies of the inter-American system are fully aware of the situation, and in light of it three of them--one responsible for agricultural development, another for health, and the other for scientific and technological development--have formed an inter-American study group on advances in the area of biotechnology. The publication at hand presents the outcome of that group's focus on the important subject of regulating DNA work. The three regional agencies have combined their efforts with a fourth international agency responsible for animal health that is likewise

interested in this subject. Finally, the regional system's banking institution, the Inter-American Development Bank, has made possible the publication of this volume, setting an example, hereby, of what can be done when agencies work together to make the most of their collective energies.

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PREFACE

In recent years biotechnology has become the main subject of meetings, symposia, conferences and reports as the different sectors of society are aware of the benefits that can accrue from the development of capabilities in this area. Biotechnology offers valuable elements for the efficient utilization of a wide variety of renewable and nonrenewable resources in industrialized societies, as well as developing countries which have natural resources suitable as raw material for the development of biological industrial processes. Recent advances in cell biology, molecular genetics, biochemistry and bioengineering have impelled the growth of a "new biotechnology." The foremost development in this new biotechnology is the rDNA (recombinant deoxyribonucleic acid) technology, from which has emerged products such as a hepatitis-B vaccine produced by yeasts, a vaccine against pseudorabies in swine, and interleukine II from Escherichia coli. New discoveries in research with rDNA and hybridomes have, at the same time, led to products such as food additives, drugs, biologic substances and medical devices. Also, the use of rDNA technology allows the introduction of greater concentrations of reserve protein into soybeans to obtain a higher nutritive product; the preparation of new microbial pesticides and ore-leaching microbes; and, many other products of the future.

The processes and products of this new biotechnology are so diverse and have so little in common that it is hard to make valid generalizations about them, irrespective of their Intended use. Therefore, for regulatory supervision biotechnology has no systematic unifying features on which any single comprehensive law could be based. The diversity of its applications and products is important as it determines that the regulation of so many final uses must be controlled by several government entities. One main issue of concern in regulation of the new biotechnology is the release of new organisms into the environment. This regulation clearly demands an examination of every new microorganism created by genetic engineering techniques before its release, though the examination can be abbreviated for organisms that are obviously harmless.

The Governments in the Region are fully cognizant of this situation and in partucular their Ministers of Agriculture expressed this awareness at the IX Inter-American Conference of Ministers of Agriculture in Ottawa, Canada, in August-September 1987. On that occasion, after a wide-ranging discussion of the subject, the Ministers decided to recommend "that the governments of Latin America and the Caribbean recognize the importance of new scientific and technological developments deriving from progress in the field of biotechnology and implement policies and legal and institutional mechanisms which make it possible to reap the benefits of these advances, consistent with the development priorities of each country, and in compliance with minimum requirements of safety for human health and environmental protection." They also recommended that the agencies of the Inter-American System, and Inter-American Institute for Cooperation on Agriculture (IICA) in particular, start working immediately with other international agencies concerned with scientific and technological development, toward the unification of standards and the proposal of policies for the design, protection and control of technologies and products emerging from advances in biotechnology.

This subject was also discussed at the First Meeting of the Regional Directing Council of the Regional Biotechnology Program, UNDP/UNESCO/ONUDI, held in Mexico, March-April 1987, at which time it was decided so: "Request the Pan American Health Organization (PAHO) to collect and distribute, to the Member Countries, basic information regarding aspects related to safety in the handling of biologic products and techniques (biosafety), with the purpose of stimulating an open discussion to define policies on said aspect." The RDC's request was supported by the Health Advisory Research Committee and by PAHO's Directing Council at their August and September 1987 meetings, respectively.

Acting on these recommendations, IICA, PAHO/WHO, the OAS, and the OIE have joined to form a Study Group consisting of scientists from the Region and experts from agencies concerned with biotechnology, to examine and discuss aspects of the use of the new technologies and their impact on the productive structure of the Region, as well. Some of the constraints of its full utilization by the systems involved in the generation and transfer of technology in the Region.

This Study Group* held its first meeting at IICA headquarters in San Jose, Costa Rica, January 26-29, 1988, to discuss general aspects on advances obtained by the new biotechnology in the areas of agriculture,.

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* See Annex I

veterinary medicine and human health, as well as specific aspects regarding the use and safety of genetic engineering techniques. They also considered a proposed draft "Guidelines for the Use and Safety of Genetic Engineering Techniques or Recombinant DNA Technology" drawn up by IICA with the collaboration of scientists of the USDA (APHIS/ARS)** on the basis of current legislation and experience in the United States, Canada, Australia, Japan and the countries of the Economic European Community.

In addition to reviewing and approving said guidelines, and having examined and discussed various aspects and subjects related to the development of biotechnology in the Region, the study group at this meeting formulated a set of recommendations for future action in this field.

Firstly, the Group felt that in order to supervise the safety problems implied by the development, production and use of new biological organisms, it was necessary and extremely important that the countries in the Region implement the proposed Guidelines and set up national technical advisory committees on biosafety (NTACBs) as focal points of the system for regulation and control of activities in recombinant DNA technology.

To continue these efforts initiated by IICA/PAHO in the biological safety area, the Group proposed setting up a permanent technical secretariat for biotechnological safety with the following objectives: (a) to make available to those responsible for cooperative programs in the area of biotechnology in the different agencies, all materials presented in the Study Group's meeting as well as future ones. These materials should also be sent to the National Biotechnology Commissions and National Science and Technology Councils; (b) to serve as a focal point for information and dissemination of new safety measures in the countries of the Region; (c) to coordinate the establishment of two data bases on standards and regulation of recombinant DNA and biosafety, one in IICA for the agriculture area and the other in PAHO for the health area, and to serve as the center for dissemination of the compiled information, and (d) to provide support and advisory services on biotechnology to national biosafety committees on request.

** USDA - United States Department of Agriculture
APHIS - Animal and Plant Health Inspection Service
ARS - Agriculture Research Service

It was further considered that these measures should be continued and expanded, and that work should begin as soon as possible on the compilation of information and preparations of guidelines on:

A. New technologies other than recombinant DNA.

B. Guidelines for the large-scale use of rDNA technology.

C. The release into the environment of organisms and products modified and produced by rDNA technology.

D. The transportation and introduction into the countries of genetic materials taking into consideration international standards and laws, as well as national standards and regulations of each country.

The Group also reviewed the various initiatives being developed in support of biotechnology in Latin America and the Caribbean, and recommended the establishment of mechanisms for coordination mechanisms to make the most of the available resources. It was recommended that (a) contacts be established with the UNIDO/WHO/UNEP working group on biosafety to exchange information, avoid duplication of effort, and cooperate in training of personnel in charge of implementing guidelines and regulations in biotechnology, and (b) the UNDP/UNIDO/UNESCO regional project be requested to hold an international meeting to discuss intellectual property rights in biotechnology, and in recombinant DNA technology in particular.

Finally, in view of the work being done by IICA in most Latin American and Caribbean countries, the nature of the work to be done in the area of biotechnology, and the economic and financial difficulties troubling the countries in the Region, it was recommended that the Institute, through its Program for the Generation and Transfer of Technology, initiate a network of centers of excellence to work in the field of agricultural biotechnology, to stimulate and support exchange of information, and to maximize the use of human and financial resources available in the countries. It was emphasized, however, that this network should not duplicate the work of other organizations or international agencies, with which IICA will have to cooperate closely.

These recommendations , formulated by the Study Group in conclusion of its proceedings are at present to be considered and approved by the organizing agencies of the meeting and their directing bodies. However, they convey the magnitude of the work yet to be done to build a modern regulatory framework for effective regulation of these new technologies, ensuring that their great potential benefits may be utilized

and the required safeguards established for the protection of human health and the environment. In addition, they express a widespread desire to cooperate among the national and international agencies concerned with the subject which is particularly encouraging given the nature of the problem and the funding requirements involved. These "Guidelines for the Use and Safety of Genetic Engineering Techniques or Recombinant DNA Technology," published with financial support from the Inter-American Development Bank (IDB), are the first product of these cooperative efforts and should not be viewed as definitive, but rather as a working document subject to review, expansion and amendment as practical experience is obtained regarding its application in the countries of the Region.

Dr. Eduardo E. Trigo

Dr. Pedro N. Acha

I. INTRODUCTION

In recent years, developments in "biotechnology" have become the subject of much attention, as different sectors of society have become aware of the possibilities of recent refinements and extensions of the various techniques. An explosion of knowledge in microbiology, molecular biology, cellular biology, biochemistry, and genetics has fueled commercial interest and expanded research and development of new products. These, in turn, promise improvement in the quality of life for both the developed and developing nations. It should be emphasized that the spectrum of available tools represents an evolving and expanding continuum that includes conventional methods of selections and breeding, mutagenesis, recombinant DNA (rDNA) techniques, cell fusion techniques, and others. While much attention has been focused on the methods used to modify organisms, it is the products of these technologies and the uses to which they will be put that should be the objects of attention, rather than the particular techniques employed to achieve those ends. However, it should be recognized that various aspects of the manufacturing process, for example, the source of substrata (malignant cell lines, human plasma) or the use of carcinogenic solvents, may elicit concerns or dictate the use of certain quality control methods.

Rational and appropriate application of the newest techniques to the solution of problems in health, food production, energy and the environment has generated technologies that have had a tangible impact in the industrialized countries. Examples include the existence of monoclonal antibody-based diagnostic kits that detect the presence of anti-HIV antibodies in blood products; second-generation pseudorabies and Hepatitis B vaccines; enzymes and other proteins for therapeutic products and for food processing; and DNA probes for detecting infectious agents and for in utero diagnosis. Literally hundreds of products are being tested, for medical, agricultural, and environmental applications.

The biological revolution resulted from many years of support for basic biological and biomedical research. However, it is very clear that the new

techniques offer powerful approaches to the solution of health, agricultural and environmental problems of all the world. As the biological technologies developed, the range of potential products expanded rapidly and raised questions about the regulation of biological research and products. During the past decade, many countries and international organizations have gained much experience with regulation of this research. As these technologies now spread rapidly throughout the hemisphere, it is important that the countries of the region learn not only the scientific techniques and approaches but also learn from the past decade of oversight. This document is intended to provide scientists and political leaders of the Region with an introduction to the issues regarding the appropriate regulation of modern biotechnology (including genetic engineering) -- regulation built on past experience and attempting to provide a scientific balanced approach to implementation and control.

For the foreseeable future, the fruits of the new biotechnology are likely to remain minuscule compared to the more than US$100 billion in annual sales of conventional products (agriculture, microbial fermentation, and so forth) derived from biotechnology.

The processes and products of this new biotechnology are so diverse and have so little in common that it is difficult to make valid generalizations about them, for whatever purpose. For purposes of regulatory oversight, the products and processes of new biotechnology have no unifying, systematic features on which homogeneous, comprehensive regulation could be based. Moreover, the mandates and areas of experience and competence of different regulatory agencies are as various as the characteristics and intended uses of the products regulated. This is not to say that oversight is impossible or difficult; rather, it implies that regulation should be based on rational and appropriate categories based on the intended use of products.

One area of concern has been the agricultural and environmental applications of organisms genetically manipulated with new techniques. There exists wide consensus that the new techniques should be regarded as refinements or extensions of older techniques, and the primary consideration is the characteristics of the product or products which are being introduced into an ecosystem and not always the method of construction of genetically modified organisms. Especially for agricultural and environmental applications of organisms, whether genetically modified or not, there is a need for classifying different organisms and establishing biological categories.

The new biotechnology has developed such that education of government regulators, the media, and the public is desirable to provide perspective on regulations that will protect human health and the environment while permitting research and development. These regulations must not be conservative but pragmatic, and must afford safety without restrictiveness. The developed countries have in place regulatory mechanisms that generally prevent the introduction or use of harmful products, regardless of their method of manufacture. In the international sphere, various groups of scientists and/or regulators have published statements of policy or principles related to the supervision of the new biology, such as for example, the Organization for Economic Development and Cooperation (OECD), Paris, 1986, and the Working Group on Biotechnology Safety of UNIDO/WHO/UNEP, Paris, December, 1987.

There is wide consensus that guidelines, regulations and legislation should be appropriately generic, and that regulatory categories should be based on scientific principles. In a number of countries, many products of the new biotechnology have been regulated successfully under pre-existing regulatory programs. The record appears to justify this policy: in the more than ten years that the new techniques have been used in the laboratory and applied in industry and agriculture, not one major safety problem referable to the new techniques has been reported.

It is critically important that regulators act quickly and decisively to counterbalance the extreme claims of those opposed blindly to the uses of the new biotechnology. Especially, if it is not intended that the new biotechnology become one more tool for aggression on the environment to be added unfortunately to the ones already existing. Certainly, it is not true that all new products are too dangerous to be introduced into the environment. This notion is refuted both by theory and by experience. Similarly, one must reject generic safety arguments; each introduction of an organism, whether modified or not, must be judged on its own merits, within the context of the scale of the application and the possible environmental and societal costs and benefits. Some well-circumscribed trials of known hazardous agents -- for example, tests of control agents for plant or animal pathogens -- will be appropriately carried out under controlled conditions. Our guideposts must be, on one hand, the knowledge that there is a genuine cost of not testing or delay in approving new products; we suffer enormous crop losses caused by freezes and are forced to rely on chemical pesticides while the "ice-minus" bacteria and new biological pesticides have languished untested and unused. At the same time, concerns of the public are legitimate and understandable. The principles that govern the safe use of various classes of products and

various processes must be refined so that basic research, development and commercialization may proceed; the stakes are too high for society not to.

In the region of the Americas great things are expected from the new biotechnology, which can open up new horizons and offer short-term solutions to severe economic and social problems. Governments are taking an interest in programs of genetic engineering and rDNA technology. Guidelines and recommendations are needed to help them prepare proper legislation for scientific investment and development.

This technology is developing by leaps and bounds, and the scientific underpinnings of its present regulation must be reviewed at regular intervals and the necessary adjustments made in its framework and in the essential characteristics thereof.

It is to be hoped that these guidelines *may* provide *a coherent framework for regulation of the new biotechnology in each of the countries and permit the safe and rational development of its scientific and material benefits, while at the same time ensuring responsibilities for the protection of health and the environment.*

The group opted for a sequential approach to ensure the adequacy of regulation. They drafted or adapted recommendations related to the safety of the uses of various product areas, focusing at this time on those produced with rDNA techniques ensuring that they are adequately addressed. The next effort will address more generally or broadly the entire continuum of "conventional" and "new" biology. Subsequent studies are planned to include such projects as large scale (industrial) guidelines and the introduction of biotechnology products to the environment.

II. BIOSAFETY CONSIDERATIONS AND DEFINITIONS

II.A NATIONAL BIOSAFETY AND TECHNICAL ADVISORY COMMITTEE (NBTAC)

The recombinant DNA techniques (rDNA) permit a wide range of genetic manipulations which were not previously feasible. These new techniques offer many possibilities for new and beneficial products, however, they have also raised new questions about safety. Governments in many countries established Recombinant DNA Advisory or Monitoring Committees. Such groups contribute to the evaluation of whether new biological hazards are in fact associated with genetic manipulation and development and administration of guidelines appropriate to the level of assessed risk and benefits.

The primary objectives are that good laboratory and manufacturing practices are established in all organizations using rDNA techniques. The guidelines should afford protection to individuals, the community and the environment, by minimizing potential hazards associated with new applications and by facilitating the beneficial utilization of the new technologies.

II.A.1 TERMS OF REFERENCE FOR THE NBTAC

Having regard to the Government's wish for a voluntary system for technical and biosafety advice to be provided to the authorities on the continuing assessment of the risk and benefits, associated with the production and/or application of biological materials produced in laboratories and which occur in nature, the Committee shall:

II.A.1.1 Establish and review, as necessary, Guidelines for both physical and biological containment and/or control procedures appropriate to the level of assessed risk involved in relevant research, development and application of rDNA biotechnology.

II.A.1.2 Review relevant proposals, except those that relate to research performed under contained laboratory conditions, and recommend any conditions under which this work should be carried out, or that the work not be undertaken.

II.A.1.3 Consult with relevant government agencies and other organizations as appropriate.

II.A.1.4 Report to the responsible governmental authorities at least annually, and also report promptly after any breaches of the Guidelines referred to in A.1.1 above, and on other relevant matters referred to them (NTACBs).

II.A.1.5 Establish contact and maintain liaison with such monitoring bodies in other countries and with international organizations, as is appropriate.

II.A.1.6 As necessary, advise on the training of personnel with regard to safety procedures.

II.A.1.7 Collect and disseminate information relevant to the above, having due regard to the special circumstances relating to proprietary information.

II.A.1.8 Establish and oversee the work of a Scientific Sub-Committee, whose guidelines follow and whose role and function include not only participation in II.A.1.3, II.A.1.5, II.A.1.6 and II.A.1.7 but also, all research performed under contained laboratory conditions.

II.A.2 THE SCIENTIFIC SUB-COMMITTEE SHALL:

II.A.2.1 Be formed to support the work of the NBTAC. Enter into discussions directly with scientists and the institutions where they work, and with fund-granting bodies in determining the conditions under which research should be carried out.

II.A.2.2 Review proposals for such research and recommend any conditions under which experiments should be carried out, or that work not be undertaken.

II.A.2.3 Provide technical advice to the NBTAC and contribute to its functions in relation to laboratory contained research.

Guidelines rely heavily on the effective operation of the institutional biosafety committee (IBC) and the biological safety officer (BSO) which should exist in all institutions working with rDNA technology in each of the countries.

Establish an IBC to provide local surveillance of Recombinant DNA work, who at the same time will appoint a BSO. The NBTAC will give both the IBC and the BSO the authority and support required to perform their functions and ensure that both are operating effectively.

II.B INSTITUTIONAL BIOSAFETY COMMITTEE (IBC)

Institutions carry the prime responsibility for the safety of employees, researchers, and the surrounding community. In this regard, institutional biosafety committees play a central role.

The membership of an IBC should include members with the appropriate expertise, and could also include external experts and lay members. Typically, IBCs include microbiologists, biologists and persons familiar with genetic techniques, the appropriate equipment and containment.

The IBC should have enough scientific members so that it is not totally dependent on the advice of a project supervisor to make assessments of that supervisor's projects. The organization may also wish to consider the inclusion of persons with a broader and not necessarily technical background. In addition, the NBTAC considers it desirable to appoint some persons from outside the organization.

It is recognized that some organizations, particularly smaller ones, may have difficulties in setting up an IBC with the requisite breadth of expertise, in which case the NBTAC is willing to provide advice and assistance. There could, for example, be a number of organizations which rely on neighbouring organizations with a well-established IBC for some or all of their surveillance.

II.B.1 TERMS OF REFERENCE AND MODE OF OPERATION OF AN IBC

The terms of reference and mode of operation of an IBC may vary, but major responsibilities in all cases are:

a) to review and endorse applications,

b) to consult with and request approvals from NBTAC if required,

c) to implement the recommendations of the NBTAC,

d) to establish a program of inspections to ensure that the physical containment facilities continue to meet requirements and that the other procedures and practices specified in these Guidelines are followed,

e) to ensure that all personnel involved in the work have sufficient training and experience,

f) to maintain a list of project supervisors and other supervisors approved by the IBC as competent to perform supervisory duties for particular projects,

g) to maintain individual records and files of individual research projects,

h) to investigate and report promptly to the NBTAC all accidents, unexplained absences and illnesses; and

i) to provide an annual report to the NBTAC.

The terms of reference of the IBC and a list of its members together with their relevant qualifications and experience should be sent to the NBTAC and should be widely circulated within the Institution.

II.B.2 ACCOUNTABILITY AND SUPERVISION

In instances, where an NBTAC or similar committee is not formed, governments have the responsibility to arrange for a technically appropriate supervisory mechanism. In some cases, existing government regulatory agencies or other groups can fulfill this important function.

II.C **BIOLOGICAL SAFETY OFFICER**

The BSO should be familiar with the biosafety requirements for the recombinant DNA work and facilities, and be able to make checks and advise on biosafety issues on a day-to-day basis. The officer should be given sufficient independence and authority to ensure that biosafety is not compromised by other considerations. The officer may be a member of the IBC. A report from the officer should form part of the IBC's annual report.

II.D PROJECT SUPERVISION

For each project there should be a Project Supervisor responsible for all aspects of the work. This person should take responsibility for fully describing the process in the Proposal Form and ensuring that the Operating Manual is accurate and deals adequately with safety and emergency procedures. The Project Supervisor must also ensure that all workers are suitably trained for the tasks they will perform and in safety and emergency procedures. Workers must be familiar with any hazards in the work area and be informed of the purpose of these Guidelines.

The Project Supervisor, and indeed all persons who will at some time supervise the work, must be approved by the IBC as having the requisite competence. The IBC should maintain a list of approved supervisors. If the Project Supervisor is changed, the IBC should be advised promptly.

III. REVIEW OF PROPOSALS AND CERTIFICATION OF FACILITIES

III.A A NOTE ON PROPRIETARY INFORMATION

The Committees should be conscious of the need to protect information which may have commercial significance. If organizations provide information which is not in the public domain and they wish to restrict access to the information, then they should mark the relevant pages "commercial-in-confidence". In these cases, organizations are requested to provide a short (less than one page) summary of the proposal which can be made public and used in annual reports.

Members of the NBTAC, its Scientific Subcommittee and local IBCs, will sign agreements of confidentiality binding them not to divulge commercial-in-confidence information. Persons who are not public servants and who are assisting in inspections will be required to sign similar agreements of confidentiality.

When visits to or inspections of facilities are being arranged, the organization concerned may request that an individual be excluded from the inspection where it can be shown that there is likely to be a conflict of interest.

III.B GENERAL COMMENTARY

The IBC and, if necessary, the NBTAC, will consider each proposal on a case-by-case basis. It will be for the organization to demonstrate to the Committee that the facility, equipment and operational practices are safe and meet the provisions of these Guidelines.

The NBTAC should be prepared to consider cases put by IBCs for the Guidelines to be varied given the particular characteristics of a specific project. It will be for the IBC to demonstrate that the proposed variations do not compromise safety. Similarly, the NBTAC envisages that the characteristics of some projects may warrant special conditions over and above those specified in these Guidelines.

III.C PRELIMINARY ADVICE FROM THE NBTAC

During the planning phase of a project the IBC may want to discuss with the NBTAC any aspects of the proposed work. Indeed, organizations are urged to consult early with the NBTAC about the likely level of physical containment and the specifications for the equipment and facilities, if there

are concerns. Such discussions should minimize difficulties or disagreements arising during the actual project.

III.D SUBMISSION OF PROPOSAL

III.D.1 PROJECT SUPERVISOR

For all work which falls within the scope of these Guidelines the Project Supervisor should complete a Proposal Form and submit it to the IBC. It should be noted that, in addition to providing information about the project, the Proposal Form requires the project supervisor to record an assessment of the safety precautions which should be followed.

In completing the Proposal Form, Project Supervisors should note that the primary concern is to ensure the safety of the proposed work. Thus, information of commercial sensitivity need not necessarily be provided. Where such material must be provided in order for the IBC or NBTAC to make a reasonable evaluation, the information should be labelled as confidential.

III.D.2 INSTITUTIONAL BIOSAFETY COMMITTEE

The IBC should review the Proposal Form and record its evaluation of the proper levels of biological and physical containment together with any required special safety conditions. It should also check that adequate arrangements have been made for supervision, training and record keeping. At that point the IBC should decide if consultation with the NBTAC is required. At any rate, the IBC should at least report annually to the NBTAC.

III.E REVIEW BY THE NBTAC

Upon request of the IBC, or if the NBTAC judges it necessary, the NBTAC will:

a. review the assessments made by the project supervisor and the IBC;

b. evaluate the required levels of physical and biological containment;

c. consider the need for any special conditions;

d. check that the Operating Manual is complete and adequately covers the safety and emergency aspects of concern, and

e. arrange an inspection, if necessary.

Following this assessment, the NBTAC will advise the IBC of its recommendations, if any. These recommendations should be implemented before work begins.

III.F INSPECTION AND CERTIFICATION OF FACILITIES

III.F.1 NEW OR MODIFIED FACILITY

For a new facility, or after significant modifications to an existing facility, the IBC will carry out an inspection. Work may not begin until the IBC has certified the facilities and, as specified above, all recommendations have been implemented. Further inspections should be made periodically. Safety and operating manuals should be prepared and all documentation and information kept up-to-date which is consistent with national and international experience.

For inspections the IBC will appoint a team of experts. This team will normally consist of a microbiologist and an expert in physical containment. Organizations should note that the inspection team will not only examine the building and the equipment but also such documents as the emergency plans and the Operating Manual.

All people participating in inspections will have signed agreements of confidentiality or be public servants. The IBC will provide organizations with the names of the inspection team in advance. Organizations may request that a particular person be excluded from the inspection where it can be shown that there would be a conflict of commercial interest, or for other valid reasons.

III.F.2 NEW PROCESS IN ALREADY CERTIFIED FACILITY

Where an organization intends to use the same facility and equipment for a number of projects, a prior inspection may only be necessary before the facility/equipment is first used for recombinant DNA work. For subsequent projects, which do not involve significant changes to the facility or equipment, the IBC should confirm that:

a. The proposed changes in the operation of the facility and equipment are minor,

b. Safety is not compromised by the changes, and

c. The new operating manual is comprehensive and adequately deals with safety and emergency aspects.

The IBC should make periodic inspections of all facilities.

IV. CONTAINMENT PRACTICES AND SPECIFICATIONS

IV.A **BIOLOGICAL CONTAINMENT (see Appendix E)**

Biological containment refers to the use of those organisms and/or vectors that have been genetically altered so that they have little chance of surviving and reproducing except under special artificial conditions. Certain host-vector systems have been certified as providing biological containment.

Organizations are encouraged to design processes incorporating biological containment and to work with systems which are well characterized (e.g. donor DNA, recipient organism). Characteristics (e.g. genetic) of organisms used in large scale work should be tested regularly. The procedures to be used for these tests are to be documented and recorded.

IV.B PHYSICAL CONTAINMENT (see Appendix D)

Physical containment refers to the use of special buildings, equipment and procedures to prevent the escape of organisms. There are basic practices which should apply to all large scale processes involving microorganisms. In many cases such procedures are covered by accepted Good Practice Codes or Guidelines, e.g.:

IV.B.1 MINIMUM PRACTICES

-In a controlled area facility:

a. the area must be kept neat and clean;

b. process equipment must be designed to minimize the potential for rupture and to permit easy decontamination and maintenance:

c. the area must be designed to contain spills in the event of a total rupture of the systems;

d. work surfaces and floors in the area must be decontaminated regularly; and

e. hand-washing facilities must be in, or next to, the area.

-Personnel practices

a. access to the work area must be controlled so that persons not involved in the process cannot inadvertently enter the area;

b. appropriate protective clothing must be worn;

c. before leaving the area or whenever contaminated with fluids from the process, hands must be washed with soap and warm water and protective clothing replaced;

d. there must be no eating, drinking, smoking, storage of food or drinks and application of cosmetics in the area;

e. such procedures as mouth pipetting are prohibited; mechanical devices must be used; and

f. workers should avoid contact with any contaminated material.

-Signs

a. a sign at the work site should indicate when work is in progress and at what level of physical containment; and

b. emergency procedures must be displayed prominently within the area.

IV.C PRIMARY CONTAINMENT

IV.C.1 PRIMARY CONTAINMENT SYSTEM

IV.C.1.1 Cultures of viable organisms or cells (including those containing recombinant DNA molecules) shall be handled in a closed system (e.g. closed vessel used for propagation, growth and storage, and closed lines used for transfer or aeration) or other primary containment equipment (e.g. biological safety cabinet containing a centrifuge used to process culture fluids) which is designed to reduce the potential for escape of viable organisms. ,

IV.C.1.2 The equipment used for the propagation and harvesting of organisms shall be checked regularly for the integrity of containment.

IV.C.2 HANDLING OF CULTURE FLUIDS

IV.C.2.1 Culture fluids (except as allowed in C.2.2) shall not be removed from a closed system or other primary containment equipment unless the viable organisms have been rendered non-viable by a procedure which has been demonstrated to be effective.

IV.C.2.2 Where the process demands the collection of viable organisms from a closed system, the addition of materials to a closed system or the transfer of culture fluids from one closed system to another, such procedures shall be done in a manner which prevents the release of aerosols from the system or contamination of exposed surfaces.

IV.C.3 FILTRATION OF EXHAUST GAS

IV.C.3.1 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 minimize the release of viable organisms.

IV.C.4 DECONTAMINATION PROCEDURES

IV.C.4.1 A closed system, or other primary containment equipment that has held viable organisms shall not be opened (e.g. for maintenance) unless it has been decontaminated by a validated procedure.

IV.C.5 EMERGENCY PROCEDURES

IV.C.5.1 Emergency procedures and the design of closed systems should be adequate to handle safely any losses of culture material in the event of partial or total rupture of the primary physical containment.

IV.C.6 SIGNS

IV.C.6.1 The universal biohazard sign must be posted on each entry.

In addition to the above practices and conditions, it is recommended that detailed guidelines be consulted for high levels of containment or for use of particularly dangerous categories of organisms.

(*) High Efficiency Particulate Air.

V. PLANNED RELEASE

V.A DEFINITION

A planned release is considered to be any experimental trial or commercial product which involves, or may involve the use of live organisms:

i. in open fields, paddocks and natural ecosystems;

ii. in enclosed facilities (e.g. shade houses, animal pens) which are not certified as to "containment".

iii. for animal or human use or consumption.

This definition is intended also to encompass work which is not intended for release as such, but which is to be performed in non-contained facilities or restricted field locations as this work may allow inadvertent releases to the environment.

V.B ASSESSMENT

The IBC (or if required, the NBTAC) should assess any project which involves the planned release of any organisms not generally accepted to be harmless. Institutions should not assume that an institutional assessment is an exemption from any or all other government regulations. Moreover, special considerations should be given to consultations on organisms likely to travel across national borders including possible transport through common aquatic systems.

V.C WORK OUTSIDE OF THE GUIDELINES

The following categories of work are either outside the scope of these guidelines or are regarded as not having any potential hazard from the recombinant DNA perspective. Other government regulatory agencies may, however, still have a need to assess these categories of work. Organizations should not assume that an exemption from assessment is an exemption from any or all other government regulations.

V.C.1 OTHER TECHNIQUES FOR GENETIC MANIPULATION

Where the genetic manipulation of cells or organisms does not use hybrid DNA molecules formed by recombinant DNA techniques, the work falls outside the scope of these terms of reference. One example of such work is cell fusion techniques.

Should an organization or a regulatory agency seek advice on the genetic aspects of one of these other genetic manipulation techniques then the IBC or NBTAC will respond if it is within its capabilities to do so.

V.C.2 SELF-CLONED ORGANISMS

Self-cloned organisms are those where the inserted or donor DNA (genetic material) is derived from the host species or from a species which is known to transfer DNA to the host species by natural physiological mechanisms. It is well established that species by nature change their genetic make-up, and that many micro-organisms in nature exchange genetic material. Where it is proposed to use the recombinant DNA technique to achieve what is known to occur in nature the NBTAC does not consider that any new hazards are associated with the work.

V.C.3 ISOLATED RECOMBINANT DNA PREPARATIONS

Work with preparations of isolated recombinant DNA molecules which do not contain viable organisms does not require review. If the preparations contain DNA which in itself is known to be infectious or otherwise harmful, conditions for handling the DNA should be related directly to the hazard associated with that of the parent organism.

V.C.4 HIGH-HAZARD ACTIVITIES

Certain experiments on organisms (e.g. with dangerous pathogens) may be considered as too dangerous for work at a given institution or facility. In all high-risk work it is recommended that the Institution seeks specific authorization for its conduct from the appropriate government authority.

VI. GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES

VI.A SCOPE OF THE GUIDELINES

VI.A.1 PURPOSE

The purpose of these Guidelines is to specify practices for constructing and handling (i) recombinant DNA molecules and (ii) organisms and viruses containing recombinant DNA molecules.

VI.A.2 RECOMBINANT DNA MOLECULES: DEFINITION

In the context of these Guidelines, recombinant DNA molecules are defined as either (1) molecules which are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) DNA molecules that result from the replication of those described in (1) above.

Synthetic DNA segments likely to yield a potentially harmful polynucleotide or polypeptide (e.g. a toxin or a pharmacologically active agent) shall be considered as equivalent to their natural DNA counterpart. If the synthetic DNA segment is not expressed in vivo as a biologically active polynucleotide or polypeptide product, it is exempt from the Guidelines

VI.A.3 GENERAL APPLICABILITY

The Guidelines are applicable to all recombinant DNA research within the Americas.

An individual receiving support for research involving recombinant DNA must be associated with or sponsored by an institution that can and does assume the responsibilities assigned in these Guidelines.

VI.A.4 GENERAL DEFINITIONS

The following terms, which are used throughout the Guidelines, are defined as follows:

VI.A.4.1 "Institution" means any public, private or international entity (including Federal, State, and a local government agency).

VI.A.4.2 "Institutional Biosafety Committee" or "IBC" means a committee that (i) meets the requirements for membership specified in Section II.B,

and (ii) reviews, approved, and oversees projects in accordance with the responsibilities defined in Sections II.B.1.

VI.A.4.3 National Biosafety and Technical Advisory Committee (NBTAC) means the public advisory committee that advises the corresponding authorities concerning recombinant DNA research. Terms of reference are described in Section II.A.1. The NBTAC is referenced from here on in these guidelines by the abbreviation RAC (Recombinant Advisory Committee).

VI.B CONTAINMENT

Effective biological safety programs have been operative in a variety of laboratories for many years. Considerable information, therefore, already exists for the design of physical containment facilities and the selection of laboratory procedures applicable to organisms carrying recombinant DNAs. The existing programs rely upon mechanisms that, for convenience, can be divided into two categories: (i) A set of standard practices that are generally used in microbiological laboratories; and (ii) special procedures, equipment, and laboratory installations that provide physical barriers which are applied in varying degrees according to the estimated biohazard. Four biosafety levels (BL) are described in Appendix D. These biosafety levels consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities appropriate for the operations performed and the hazard posed by agents and for the function and activity of the laboratory. Biosafety level 4 (BL4) provides the most stringent containment conditions; BL1 the least stringent.

Experiments on recombinant DNAs by their very nature lend themselves to a third containment mechanism -- namely, the application of highly specific biological barriers. In fact, natural barriers do exist which limit either (i) the infectivity of a vector or vehicle (plasmid or virus) for specific hosts, or (ii) its dissemination and survival in the environment. The vectors that provide the means for replication of the recombinant DNAs and/or the host cells in which they replicate can be genetically designed to decrease by many orders of magnitude the probability of dissemination of recombinant DNAs outside the laboratory.

As these three means of containment are complementary, different levels of containment appropriate for experiments with different recombinants can be established by applying various combinations of the physical and biological barriers along with a constant use of the standard practices. We consider these categories of containment separately in order that such combinations can be conveniently expressed in the Guidelines. (Appendix D and E).

In constructing these Guidelines, it was necessary to define boundary conditions for the different levels of physical and biological containment and for the classes of experiments to which they apply. We recognize that these definitions do not take into account all existing and anticipated information on special procedures that will allow particular experiments to be carried out under different conditions than indicated here without affecting risk. Indeed, we urge that individual investigators devise simple and more effective containment procedures and that investigators and IBCs recommend changes in the Guidelines to permit their use.

VI.C GUIDELINES FOR CATEGORIZED EXPERIMENTS

Part VI.C discusses experiments involving recombinant DNA. These experiments have been divided into four classes:

VI.C.1 Experiments which require specific RAC review and IBC approval before initiation of the experiment;

VI.C.2 Experiments which require IBC approval before initiation of the experiment;

VI.C.3 Experiments which require IBC notification at the time of initiation of the experiment;

VI.C.4 Experiments which are exempt from the procedures of the Guidelines.

VI.C.1 EXPERIMENTS THAT REQUIRE RAC REVIEW AND IBC APPROVAL BEFORE INITIATION

Experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to the corresponding authorities, review by the RAC, and specific approval by IBC. The containment conditions for such experiments will be recommended by RAC and set by the corresponding authorities at the time of approval. Such experiments also require the approval of the IBC before initiation. These experiments may be:

VI.C.1.1 Deliberate formation of recombinant DNAs containing genes for the biosynthesis of toxic molecules lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight (e.g. microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, Shigella. dysenteriae neurotoxin). Specific approval has been given for the cloning in E. coli K-12 of genes containing DNA coded for the biosynthesis of toxic molecules which are lethal to vertebrates at 100 nanograms to 100 micrograms per kilogram body weight. Containment levels for these experiments are to be determined by RAC and approved and verified by IBC.

VI.C.1.2 Deliberate release into the environment of any organism containing recombinant DNA, except certain plants according to norms and conditions established by the agriculture authorities.

VI.C.1.3 Deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire it naturally, if such acquisition could compromise the use of the drug to control disease agents in human or veterinary medicine or agriculture.

VI.C.1.4 Deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into human subjects. The requirement for RAC review should not be considered to pre-empt any other required review of experiments with human subjects.

VI.C.2 EXPERIMENTS THAT REQUIRE IBC APPROVAL BEFORE INITIATION

Investigators performing experiments in this category must submit to their IBC, prior to initiation of the experiments, a registration document that contains a description of: (i) The sources of DNA; (ii) the nature of the inserted DNA sequences; (iii) the hosts and vectors to be used; (iv) whether a deliberate attempt will be made to obtain expression of a foreign gene, and, if so, what protein will be produced; and (v) the containment conditions specified in these Guidelines. This registration documents must be dated and signed by the investigator and filed only with the local IBC. The IBC shall review all such proposals prior to initiation of the experiments. Requests for lowering of containment for experiments in this category will be considered by the corresponding authorities.

VI.C.2.1 Experiments Using Human or Animal Pathogens (Class 2, Class 3, Class 4, or Class 5(*) Agents) as Host-Vector Systems

VI.C.2.1.a Experiments involving the introduction of recombinant DNA into Class 2 agents can be carried out at BL2 containment.

VI.C.2.1.b Experiments involving the introduction of recombinant DNA into Class 3 agents can be carried out at BL3 containment.

VI.C.2.1.c Experiments involving the introduction of recombinant DNA into Class 4 agents can be carried out at BL4 containment.

VI.C.2.1.d Containment conditions for experiments involving the introduction of recombinant DNA into Class 5 agents will be set on a

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Classification of Ehiologic Agents on the basis of Hazard. CDC/USPHS/DREW, 4 ED. 1979.

case-by-case basis following specific regulations of each country regarding handling of pathogenic agents foreign to their territory.

VI.C.2.2 Experiments in which DNA from Human or Animal Pathogens (Class 2, Class 3, Class 4, or Class 5(*) Agents) is Cloned in *Nonpathogenic Prokaryotic or Lower Eukaryotic Host Vector Systems*

VI.C.2.2.a Recombinant DNA experiments in which DNA from Class 2 or Class 3 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment. Recombinant DNA experiments in which DNA from Class 4 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes can be performed at BL2 containment after demonstration that only a totally and irreversibly defective fraction of the agent's genome is present in a given recombinant. In the absence of such a demonstration, BL4 containment should be used. Specific lowering of containment to BL1 for particular experiments can be approved by the IBC. Many experiments in this category will be exempt from the Guidelines (see Sections VI.C.4 and VI.C.5).

VI.C.2.2.b Containment conditions for experiments in which DNA from Class 5 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes will be determined by the corresponding authorities, following a case-by-case review.

VI.C.2.3 Experiments Involving the Use of Infectious Animal or Plant DNA or *RNA Viruses or Defective Animal or Plant DNA or RNA Viruses in the* Presence of Helper Virus in Tissue Culture Systems

VI.C.2.3.a Experiments involving the use of infectious Class 2 animal viruses or defective Class 2 animal viruses in the presence of helper virus can be performed at BL2 containment.

VI.C.2.3.b Experiments involving the use of infectious Class 3 animal viruses or defective Class 3 animal viruses in the presence of helper virus can be carried out at BL3 containment.

VI.C.2.3.c Experiments involving the use of infectious Class 4 viruses or defective Class 4 viruses in the presence of helper virus may be carried out under BL4 containment.

VI.C.2.3.d Experiments involving the use of infectious Class 5 viruses or defective Class 5 viruses in the presence of helper virus will be determined on a case-by-case basis by the corresponding authorities.

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* Classification of Ethiologic Agents on the basis of Hazard CDC/USPHS/DHEW, 4 ED. 1979.

VI.C.2.3.e Experiments involving the use of infectious animal or plant viruses or defective animal or plant viruses in the presence of helper virus not covered by in VI.C.2.3.a, VI.C.2.3.b, VI.C.2.3.c or VI.C.2.3.d may be carried out under BLi containment.

VI.C.2.4 Recombinant DNA Experiments Involving Whole Animals or Plants

VI.C.2.4.a Recombinant DNA, or RNA molecules derived from any source except for that from greater than two-thirds of a eukaryotic viral genome may be transferred to any non-human vertebrate organism and propagated under conditions of physical containment comparable to BL1 and appropriate to the organism under study. It is important that the investigator demonstrate that the fraction of the viral genome being utilized does not lead to productive infection.

VI.C.2.4.b For all experiments involving whole animals and plants and not covered by Section VI.C.2.4.a, the appropriate containment will be determined by the IBC.

VI.C.2.5 Experiments Involving More Than 10 Liters of Culture

The appropriate containment will be decided by the IBC. Where appropriate, Physical Containment for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules, should be used.

VI.C.3 EXPERIMENTS THAT REQUIRE IBC NOTICE SIMULTANEOUSLY WITH INITIATION OF EXPERIMENTS

Experiments not included in sections VI.C.1, VI.C.2 and VI.C.4 and subsections of these sections are to be considered in Section VI.C.3. All such experiments can be carried out at BL1 containment. For experiments in this category, a registration document as described in VI.C.2 must be dated and signed by the investigator and filed with the local IBC at the time of initiation of the experiment. The IBC shall review all such proposals, but IBC review prior to initiation of the experiment is not required.

For example, experiments in which all components derive from non-pathogenic prokaryotes and non-pathogenic lower eukaryotes fall under Section VI.C.3 and can be carried out at BL1 containment.

CAUTION: Experiments Involving Formation of Recombinant DNA Molecules Containing no more than Two-Thirds of the Genome of any **Eukaryotic Virus. Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (all viruses from a single family being considered identical) may be propagated and maintained in cells in tissue culture using BL1 containment. For such experiments, it must be shown that the cells lack helper virus for the specific families of defective**

viruses being used. If helper virus is present, procedures specified under Section VI.C.2.3 should be used. The DNA may contain fragments of the genome of viruses from more than one family but each fragment must be less than two-thirds of a genome.

VI.C.4 EXEMPT EXPERIMENTS

The following recombinant DNA molecules are exempt from these Guidelines and no registration with the IBC is necessary:

VI.C.4.1 Those that are not in organisms or viruses.

VI.C.4.2 Those that consist entirely of DNA segments from a single nonchromosomal or viral DNA source though one or more of the segments may be a synthetic equivalent.

VI.C.4.3 Those that consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species) or when transferred to another host by well established physiological means; also, those that consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).

VI.C.4.4 Certain specified recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes though one or more of the segments may be a synthetic equivalent.

VI.C.4.5 Other classes of recombinant DNA molecules, if corresponding authorities with the advice of the RAC, after appropriate notice and opportunity for public comments, find that they do not present a significant risk to health or the environment.

VI.D ROLES AND RESPONSIBILITIES

VI.D.1 POLICY

Safety in activities involving recombinant DNA depends on the individual conducting them. The Guidelines cannot anticipate every possible situation. Motivation and good judgment are the key essentials to protection of health and the environment.

The Guidelines are intended to help the institution, Institutional Biosafety Committee (IBC), Biological Safety Office (BSO), and Principal Investigator (PI) determine the safeguards that should be implemented. These Guidelines will never be complete or final, since all conceivable experiments involving recombinant DNA cannot be foreseen. Therefore, it

is the responsibility of the institution and those associated with it to adhere to the intent of the Guidelines as well as to the specifics.

Each institution (and the IBC acting on its behalf) is responsible for ensuring that recombinant DNA activities comply with the Guidelines. General recognition of institutional authority and responsibility properly establishes accountability for safe conduct of the research at the local level.

The following roles and responsibilities constitute an administrative framework in which safety is an essential and integral part of research involving recombinant DNA molecules.

VI.D.2 RESPONSIBILITY OF THE INSTITUTION

VI.D.2.1 General Information. Each institution conducting or sponsoring recombinant DNA research covered by these Guidelines is responsible for ensuring that the research is carried out in full compliance with the provisions of the Guidelines. In order to fulfill this responsibility, the institution shall:

VI.D.2.1.a Establish and implement policies that provide for the safe conduct of recombinant DNA research and that ensure compliance with the Guidelines. The institution as part of its general responsibilities for implementing the Guidelines may establish additional procedures as deemed necessary to govern the institution and its components in the discharge of its responsibilities under the Guidelines. This may include: (i) Statements formulated by the institution for general implementation of the Guidelines, and (ii) whatever additional precautionary steps the institution may deem appropriate.

VI.D.2.1.b Establish an IBC that meets the requirements set forth in *Section* II.B. and VI.D.2.2 and carries out the functions detailed in Sections II.B.1 and VI.D.2.3.

VI.D.2.1.c If the institution is engaged in recombinant DNA research at the BL3 or BL4 containment level, appoint a BSO, who shall be a member of the IBC and carry out the duties specified in Section VI.D.2.4.

VI.D.2.1.d Require that investigators responsible for research covered by these Guidelines comply with the provisions of Section VI.D.2.5 and assist investigators to do so.

VI.D.2.1.e Ensure appropriate training for the IBC chairperson and members, the BSO, principal investigators, and laboratory staff regarding the Guidelines, their implementation and laboratory safety. Responsibility for training IBC members may be carried out through the IBC chairperson. Responsibility for training laboratory staff may be carried out through the

PI. The institution is responsible for seeing that the PI has sufficient training but may delegate this responsibility to the IBC.

VI.D.2.1.f Determine the necessity in connection with each project for health surveillance of recombinant DNA research personnel, and conduct if found appropriate, a health surveillance program for the project. (The "Laboratory Safety Monograph" (LSM) Supplement USPHS/NIH, 1981) discusses various possible components of such a program -- for example, records of agents handled, active investigation of relevant illnesses and the maintenance of serial serum samples for monitoring serologic changes that may result from the employees' work experience. Certain medical conditions may place a laboratory worker at increased risk in any endeavor where infectious agents are handled. Examples given in the LSM include gastrointestinal disorders and treatment with steroids, immunosuppressive drugs, or antibiotics. Workers with such disorders or treatment should be evaluated to determine whether they should be engaged in research with potentially hazardous organisms during their treatment or illness.

VI.D.2.2 Membership and Procedures of the IBC. The institution shall establish an IBC whose responsibilities need not be restricted to recombinant DNA. The committee shall meet the following requirements:

VI.D.2.2.a Each institution will determine the IBC integration. It is recommended that at least one IBC member, belong to another official public organization competent in the subject matter. The BSO should be an IBC member. It is compulsory to designate the BSO only in institutions that work with levels BL3 and BL4.

The IBC shall comprise no fewer than five members so selected that they collectively have experience and expertise in recombinant DNA technology and the capability to assess the safety of recombinant DNA research experiments and any potential risk to public health or the environment. At least two members shall not be affiliated with the institution (apart from their membership on the IBC) and shall represent the interest of the surrounding community with respect to health and protection of the environment. Members meet this requirement if, for example, they are officials of State or local public health or environmental protection agencies, members of other local governmental bodies, or persons active in medical, occupational health, or environmental concerns in the community. The BSO, mandatory when research is being conducted at the BL3 and BL4 levels, shall be an IBC member.

VI.D.2.2.b In order to ensure the competence necessary to review recombinant DNA activities, it is recommended that: (1) the IBC include persons with expertise in recombinant DNA technology, biological safety,

and physical containment; (ii) the IBC include, or have available as consultants, persons knowledgeable in institutional commitments and policies, applicable law, standards of professional conduct and practice, community attitudes, and the environment; and (iii) at least one member be from the laboratory technical staff.

VI.D.2.2.c The institution shall identify the committee members by name in a report to RAC and shall include relevant background information on each member in such form and at such times as RAC may require.

VI.D.2.2.d No member of an IBC may be involved (except to provide information requested by the IBC) in the review or approval of a project in which he or she has been or expects to be engaged or has a direct financial interest.

VI.D.2.2.e The institution, who is ultimately responsible for the effectiveness of the IBC, may establish procedures that the IBC will follow in its initial and continuing review of applications, proposals, and activities. (IBC review procedures are specified in Section VI.D.3.3.a).

VI.D.2.2.f Institutions are encouraged to open IBC meetings to public whenever possible, consistent with the protection of privacy and proprietary interests.

VI.D.2.2.g Upon request, the institution shall make available to the public all minutes of IBC meetings and any documents submitted to or received from funding agencies which the latter are required to make available to the public.

VI.D.2.2.h The IBC will actively participate in the evaluation of the proposals for the construction of installations BL3 and BL4 in the biotechnology research laboratories, with the purpose of guaranteeing an adequate project and also control the level of containment.

VI.D.2.2.i If there were a difference of opinions over the approval of a project regarding the containment level in which the biotechnology research should be conducted, an agreement should be reached by vote of the IBC members accepting the criteria of the majority.

VI.D.2.3 Functions of the IBC. On behalf of the institution, the IBC is responsible for:

VI.D.2.3.a Reviewing for compliance with the Guidelines recombinant DNA research as specified in VI.B and VI.C, conducted at or sponsored by the institution, and approving those research projects that it finds are in conformity with the Guidelines. This review shall include:

VI.D.2.3.a-(1) An independent assessment of the containment levels required by these Guidelines for the proposed research, and

VI.D.2.3.a-(2) An assessment of the facilities, procedures, and practices, and of the training and expertise of recombinant DNA personnel.

VI.D.2.3.b Notifying the principal investigator of the results of their review.

VI.D.2.3.c Lowering containment levels for certain experiments as specified in Section VI.C.2.2.

VI.D.2.3.d Setting containment levels as specified in Sections VI.C.2.4-b and VI.C.2.5.

VI.D.2.3.e Reviewing periodically recombinant DNA research being conducted at the institution to ensure that the requirements of the Guidelines are being fulfilled.

VI.D.2.3.f Adopting emergency plans covering accidental spills and personnel contamination resulting from such research.

VI.D.2.3.g Reporting as soon as possible within a maximum of up to 30 days to the appropriate institutional official any significant problem or violation of the Guidelines and any significant accidents or illnesses related to the research of levels BL3 and BL4, unless the IBC determines that the principal investigator has done so.

VI.D.2.4 Biological Safety Officer (BSO). The institution shall appoint a BSO if it engages in recombinant DNA research at the BL3 or BL4 containment level. The officer shall be a member of the IBC, and his or her duties shall include (but need not be limited to):

VI.D.2.4.a Ensuring through periodic inspections that laboratory standards are rigorously followed;

VI.D.2.4.b Reporting to the IBC and the institution all significant problems with and violations of the Guidelines and all significant research-related accidents and illnesses of which the BSO becomes aware unless the BSO determines that the principal investigator has done so;

VI.D.2.4.c Developing emergency plans for dealing with accidental spills and personnel contamination and investigating recombinant DNA research laboratory accidents.

VI.D.2.4.d Providing advice on laboratory security;

VI.D.2.4.e Providing technical advice to the principal investigator and the IBC on research safety procedures.

VI.D.2.5 *Principal Investigator (PI).* On behalf of the institution, the PI is responsible for complying fully with the Guidelines in conducting any recombinant DNA research.

VI.D.2.5.a *General.* As part of this general responsibility, the PI shall:

VI.D.2.5.a-(1) Not initiate or modify recombinant DNA research requiring approval by the IBC prior to initiation (see Sections VI.C.1 and VI.C.2) until that research or the proposed modification thereof has been approved by the IBC and has met all other requirements of the Guidelines:

VI.D.2.5.a-(2) Determine whether experiments are covered by Section VI.C.3 and follow the appropriate procedures;

VI.D.2.5.a-(3) Report as soon as possible within a maximum of up to 30 days to the IBC and all higher authorities significant problems with and violations of the Guidelines and all significant research-related accidents and illnesses;

VI.D.2.5.a-(4) Adhere to IBC-approved emergency plans and measures for dealing with accidental spills and personnel contamination; and

VI.D.2.5.a-(5) Comply with shipping requirements for recombinant DNA molecules, established by RAC and IBC.

VI.D.2.5.b *Submissions by the Principal Investigator to the IBC.* The PI shall:

VI.D.2.5.b-(1) Make the initial determination of the required levels of physical and biological containment in accordance with the Guidelines;

VI.D.2.5.b-(2) Select appropriate microbiological practices and laboratory techniques to be used in the research;

VI.D.2.5.b-(3) Submit the initial research protocol if covered under Guidelines Sections VI.C.1, VI.C.2 or VI.C.3 (and also subsequent changes -- e.g. changes in the source of DNA or host-vector system) to the IBC for review and approval or disapproval; and

VI.D.2.5.b-(4) Remain in communication with the IBC throughout the conduct of the project.

VI.D.2.5.c *Responsibilities of the Principal Investigator Prior to Initiating Research.* The PI is responsible for:

VI.D.2.5.c-(1) Making available to the laboratory staff copies of the protocols that describe the potential biohazards and the precaution to be taken;

VI.D.2.5.c-(2) Instructing and training staff in the practices and techniques required to ensure safety and in the procedures for dealing with accidents; and

VI.D.2.5.c-(3) Informing the staff of the reasons and provisions for any precautionary medical practices advised or requested, such as vaccination or serum collection.

VI.D.2.5.d *Responsibilities of the Principal Investigator During the Conduct of the Research.* The PI is responsible for:

VI.D.2.5.d-(1) Supervising the safety performance of the staff to ensure that the required safety practices and techniques are employed;

VI.D.2.5.d-(2) Investigating and reporting in writing to RAC, the BSO (where applicable), and the IBC any significant problems pertaining to the operation and implementation of containment practices and procedures;

VI.D.2.5.d-(3) Correcting any errors and conditions in the workplace that may result in the release of recombinant DNA materials;

VI.D.2.5.d-(4) Ensuring the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g. purity and genotypic and phenotypic characteristics).

APPENDIX A - EXEMPTIONS UNDER SECTION VI.C.4

Section VI.C.4.4 states that exempt from these Guidelines are certain specified recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes though one or more of the segments may be a synthetic equivalent.

Under Section VI.C.4 of these Guidelines are recombinant DNA molecules that are: (1) Composed entirely of DNA segments from one or more of the organisms within a sublist and (2) to be propagated in any of the organisms within a sublist. (Classification of Bergey's Manual of *Determinative Bacteriology,* 8th edition. R. E. Buchanan and N.E. Gibbons editors. Williams and Wilkins Company: Baltimore, 1974).

Although these experiments are exempt, it is recommended that they be performed at the appropriate biosafety level for the host or recombinant organism (for biosafety levels see *Biosafety in Microbiological and Biomedical Laboratories,* 1st Edition (March 1984), U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Atlanta, Georgia, 30333 and National Institutes of Health, Bethesda, Maryland 20892).

Sublist A

1. Genus *Escherichia*
2. Genus Shigella
3. Genus *Salmonella* (including *Arizona)*
4. Genus *Enterobacter*
5. Genus *Citrobacter* (including *Levinea)*
6. Genus *Klebsiella*
7. Genus *Erwinia*
8. *Pseudomonas aeruginosa, Pseudomonas Putida* and *Pseudomonas fluorescens*
9. *Serratia marcescens*
10. *Yersinia enterocolítica*

Sublist B

1. *Bacillus subtilis*
2. *Bacillus licheniformis*
3. *Bacillus pumilus*
4. *Bacillus globigii*
5. *Bacillus niger*

6. Bacillus nato
7. Bacillus amyloliquefaciens
8. Bacillus aterrimus

Sublist C

1. Streptomyces aureofaciens
2. Streptomyces rimosus
3. Streptomyces coelicolor

Sublist D

1. Streptomyces griseus
2. Streptomyces cyaneus
3. Streptomyces venezuelae

Sublist E

1. One way transfer of Streptococcus mutans or Streptococcus lactis DNA into Streptococcus sanguis.

Sublist F

1. Streptococcus sanguis
2. Streptococcus pneumoniae
3. Streptococcus faecalis
4. Streptococcus pyogenes
5. Streptococcus mutans

APPENDIX B--CLASSIFICATION OF MICROORGANISMS ON THE BASIS OF HAZARD

Appendix B-I--Classification of Etiologic Agents

The original reference for this classification was the publication Classification of Etiological Agents on the Basis of Hazard, 4th Edition, July 1974, U.S. Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, Office of Biosafety, Atlanta, Georgia 30333. For the purposes of these Guidelines, this list has been revised by the National Institutes of Health, (NIH), Bethesda, MD.

Appendix B-I-A. Class 1 Agents. All bacterial, parasitic, fungal, viral, rickettsial, and chlamydial agents not included in higher classes.

Appendix B-I-B. Class 2 Agents.

Appendix B-I-B-1. Bacterial Agents.

Acinetobacter calcoaceticus
Actinobacillus -- all species
Aeromonas hydrophila
Arizona hinshawii--all serotypes
Bacillus anthracis
Bordetella--all species
Borrelia recurrentis, B. vincenti
Campylobacter fetus
Campylobacter jejuni
Chlamydia trachomatis
Chlamydia psittaci
Clostridium botulinum,
Cl. chauvoei, Cl. haemolyticum,
Cl. histolyticum, Cl. novyi,
Cl. septicum, Cl. tetani
Corynebacterium *diphtheriae,*
C. equi, C. haemolyticum,
C. pseudotuberculosis,
C. pyogenes, C. renale

Edwardsiella tarda
Erysipelothrix Insidiosa
Escherichia coli-all enteropathogenic,
enterotoxigenic, enteroinvasive and
strains bearing KI antigen
Haemophilus ducreyi, H. influenzae
Klebsiella--all species and all serotypes
Legionella pneumophila
Leptospira interrogans--all serotypes
Listeria--all species
Moraxella--all species
Mycobacteria--all species except those listed in Class 3
Mycoplasma--all species except, Mycoplasma mycoldes
and Mycoplasma agalactiae, which are in Class 5
Neisseria gonorrhoeae, N. meningiddis
Pasteurella--all species except those listed in Class 3
Salmonella--all species and all serotypes
Shigella--all species and all serotypes
Sphaerophorus necrophorus
Staphylococcus aureus
Streptobacillus moniliformis
Streptococcus pneumoniae
Streptococcus pyogenes
Treponema carateum, T. pallidum, and T. pertenue
Vibrio cholerae
Vibrio parahemolyticus
Yersinia enterocolitica

Appendix B-I-B-2. Fungal Agents.

Actinomycetes (including Nocardia species,
Actinomyces species, and Arachnia propionica) (*)
Blastomyces dermatitidis
Cryptococcus neoformans
Paracoccidioides braziliensis

(*) **The Actinomycetes have been reclassified as Bacterial Agents since the 1974 publication of the CDC/USPHS Classification of Etiologic Agents on the Basis of Hazard**

Appendix B-I-B-3. Parasitic Agents.

Entamoeba histolytica
Leishmania sp.
Naegleria gruberi
Schistosoma mansoni
Toxoplasma gondii
Toxocara canis
Trichinella spiralis
Trypanosoma cruzi

Appendix B-I-B-4. Viral, Rickettsial, and Chlamydial Agents.

Adenovirus--human--all types
Cache Valley Virus
Coxsackie A and B Viruses
Cytomegaloviruses
Echoviruses--all types
Encephalomyocarditis virus (EMC)
Flanders Virus
Hart Park Virus
Hepatitus--associated antigen material
Herpes Viruses--except Herpesvirus simiae (Monkey B Virus), which is in Class 4
Corona Viruses
Influenza Viruses--all types except A/PR8/34, which is in Class 1
Langat Virus
Lymphogranuloma venereum agent
Measles Virus
Mumps Virus
Parainfluenza virus--all types except Parainfluenza virus 3, SF4 strain, which is in Class 1
Polioviruses--all types, wild and attenuated
Poxviruses--all types except Alastrim, Smallpox and Whitepox, which are Class 5, and Monkey pox which depending *on experiments is in Class 3 or Class 4*
Rabies virus--all strains except Rabies street virus which should be classified in Class 3
Reoviruses--all types

Respiratory syncytial virus
Rhinoviruses--all types
Rubella virus
Simian Viruses--all types except Herpes virus simiae (Monkey B Virus) and Marburg Virus, which are in Class 4
Sindbis Virus
Tensaw Virus
Turlock Virus
Vaccinia Virus
Varicella Virus
Vesicular stomatititis virus
Vole rickettsia
Yellow fever virus, 17D vaccine strain

Appendix B-I-C. Class 3 Agents

Appendix B-I-C-1. Bacterial agents.

Bartonella--all species
Brucella--all species
Francisella tularensis
Mycobacterium avium, M. Bovis, M. tuberculosis
Pasteurella multocide type B ("buffalo" and other foreign virulent strains)
Pseudomonas mallei
Pseudomonas pseudomallei
Yersinia pestis

Appendix B-1-C-2. Fungal Agents.

Coccidiodes immitis
Histoplasma capsulatum
Histoplasma capsulatum, var. duboisli

Appendix B-I-C-3. Viral, Rickettsial, and Chlamydial Agents.

Monkey pox, when used in vitro
Arboviruses--all strains except those in Class 2 and 4. (West Nile and Semliki Forest viruses may be classified up or down depending on the conditions of use and geographical location of the laboratory)
Dengue Virus, when used for transmission or animal inoculation experiments

Lymphocytic choriomeningitis virus (LCM)

Rickettsia--all species except Volerickettsia when used for transmission or animal inoculation experiments

Yellow Fever Virus--wild, when *used in vitro*

Appendix B-1-D. Class 4 Agents.

Appendix B-I-D-1. Bacterial Agents.

None

Appendix B-1-D-2. Fungal Agents.

None

Appendix B-I-D-3. Parasitic Agents.

None

Appendix B-1-D-4. Viral, Rickettsial, and Chlamydial Agents.

Ebola fever virus

Monkey pox, when *used* for *transmission or animal inoculation* experiments

Hemorrhagic fever agents, including Crimean hemorrhagic fever, (Congo), Junin, and Machupo viruses, and others as yet undefined

Herpesvirus simiae (Monkey B virus)

Lassa Virus

Marburg Virus

Tick-borne encephalitis virus complex, including *Russian spring-summer encephalitis, Kyasanur forest disease, Omsk hemorrhagic fever, and Central European encephalitis viruses*

Venezuelan equine encephalitis virus, epidemic strains, when used *for transmission or animal inoculation* experiments

Yellow fever virus--wild, when *used for transmission or animal inoculation* experiments

Appendix B-II-Classification of Oncogenic Viruses on the Basis of Potential Hazard

Appendix B-II-A. Low Risk Oncogenic Viruses

Rous Sarcoma
SV-40
CELO
Ad7-SV40
Polyoma
Bovine Papilloma
Rat mammary tumor
Avian leukosis
Murine leukemia
Murine Sarcoma
Mouse mammary tumor
Rat leukemia
Hamster Leukemia
Bovine Leukemia
Dog sarcoma
Mason-Pfizer monkey virus
Marek's
Guinea pig Herpes
Lucke (Frog)
Adenovirus
Shope Fibroma
Shope Papilloma

Appendix B-II-B. Moderate-Risk Oncogenic Viruses

Ad2-SV40
FeLV
HV Saimiri
EBV
SSV-1
GaLV
HV ateles
Yaba
FeSV

Appendix B-III-Class 5 Agents

Appendix B-III-A. Animal Disease Organisms *Which* Entry is Forbidden in the Respective Legislation of *Each* Country

Example: Foot-and-mouth disease virus

Appendix B-III-B. Animal Disease Organisms and Vectors *Which* Entry is Forbidden in the Majority of the Countries in the Americas

African horse sickness virus
African swine fever virus
Besnoitia besnoiti
Borna disease virus
Bovine infectious petechial fever
Camel pox virus
Ephemeral fever virus
Fowl plague virus
Goat pox virus
Hog cholera virus
Louping ill virus
Lumpy skin disease virus
Nairobi sheep disease virus
Newcastle disease virus (Asiatic strains)
Mycoplasma mycoides (contagious bovine pleuropneumonia)
Mycoplasma agalactiae (contagious agalactia of sheep)
Rickettsia ruminatium (heart water)
Rift valley fever virus
Rhinderpest virus
Sheep pox virus
Swine vesicular disease virus
Teschen disease virus
Trypanosoma vivax (Nagana)
Trypanosoma evansl
Theileria parva (East Coast fever)
Theileria annulata
Theileria lawrencei
Theileria bovis
Theileria *hirci*
Vesicular exanthema virus
Wesselsbron disease virus
Zyonema

APPENDIX C - EXEMPTIONS UNDER SECTION VI.C.4.5

Section VI.C.4.5 states that exempt from these Guidelines are "Other classes of recombinant DNA molecules if the corresponding authorities, with advice of the RAC, after appropriate notice and opportunity for public comment finds that they do not present a significant risk to health or the environment".

The following classes of experiments are exempt under Section VI.C.4.5 of the Guidelines:

Appendix C-I--Recombinant DNAs in Tissue Culture

Recombinant DNA molecules containing less than one-half of any eukaryotic viral genome (all viruses from a single family being considered identical) that are propagated and maintained in cells in tissue culture are exempt from these Guidelines with the exceptions listed below.

Exceptions.

i) Experiments described in Section VI.C.1 require specific RAC review and IBC approval before initiation of the experiment.

ii) Experiments involving DNA from Class 3, 4 or 5 organisms or cells known to be infected with these agents.

iii) Experiments involving the deliberate introduction of genes coding for the biosynthesis of molecules toxic for vertebrates.

Appendix C-II-Experiments Involving E. coli K-12 Host-Vector Systems

Experiments which use E. coli K-12 host-vector systems, with the exception of those experiments listed below, are exempt from these Guidelines provided that: (i) the E. coli host shall not contain conjugation proficient plasmids or generalized transducing phages; and (ii) lambda or lambdoid or Ff bacteriophages or nonconjugative plasmids shall be used as vectors. However, experiments involving the insertion into E. coli K-12 of DNA from prokaryotes that exchange genetic information with E. coli may be performed with any E coli K-12 vector (e.g. conjugatioe plasmid). When a nonconjugative vector is used, the E. coli K-12 host may contain conjugation-proficient plasmids either autonomous or integrated, or generalized transducing phages.

For these exempt laboratory experiments, BL1 physical containment conditions are recommended.

For large-scale (LS) fermentation experiments BL1-LS physical containment conditions are recommended. However, following review by the IBC of appropriate data for a particular host-vector system, some latitude in the application of BL1-LS requirements is permitted.

Exceptions.

i) Experiments described in Section VI.C.1 which require specific RAC review and IBC approval before initiation of the experiment.

ii) Experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates.

Appendix C-III-Experiments Involving Saccharomyces Host-Vector Systems

Experiments which use Saccharomyces cerevisiae host-vector systems, with the exception of experiments listed below, are exempt from these Guidelines.

Experiments which use Saccharomyces uvarum host-vector systems, with the exception of experiments listed below, are exempt from these Guidelines.

For these exempt laboratory experiments, BL1 physical containment conditions are recommended.

For large-scale fermentation experiments BL1-LS physical containment conditions are recommended. However, following review by the IBC of appropriate data for a particular host-vector system some latitude in the application of BL1-LS requirements is permitted.

Exceptions.

i) Experiments described in Section VI.C.1 which require specific RAC review and IBC approval before initiation of the experiment.

ii) Experiments involving Class 3, 4 or 5 organisms or cells known to be infected with these agents may be conducted under containment conditions specified in Section VI.C.2 with prior IBC review and approval.

iii) Larger-scale experiments (e.g. more than 10 liters of culture) require prior IBC review and approval (see Section VI.C.2.5).

iv) Experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates.

Appendix C-IV--Experiments Involving Bacillus subtilis Host-Vector Systems

Any sporogenic Bacillus subtilis strain which does not revert to a sporeformer with a frequency greater than 10^{-7} can be used for cloning DNA with the exception of those experiments listed below.

For these exempt laboratory experiments, BL1 physical containment conditions are recommended.

For large-scale fermentation experiments BL1-LS physical containment conditions are recommended. However, following review by the IBC of appropriate data for a particular host-vector system, some latitude in the application of BL1-LS requirements is permitted.

Exceptions.

i) Experiments described in Section VI.C.1 which require specific RAC review and approval before initiation of the experiment.

ii) Experiments involving Class 3, 4 or 5 organisms or cells known to be infected with these agents may be conducted under containment conditions specified by Section VI.C.2 with prior IBC review and approval.

iii) Large-scale experiments (e.g. more than 10 liters of culture) require prior IBC review and approval (see Section VI.C.2.5).

iv) Experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates.

Appendix C-V--Extrachromosomal Elements of Gram Positive Organisms

Recombinant DNA molecules derived entirely from extrachromosomal elements of the organisms listed below (including shuttle vectors constructed from vectors described in Appendix C), propagated and maintained in organisms listed below are exempt from these Guidelines.

Bacillus subtilis
Bacillus pumilus
Bacillus licheniformis
Bacillus thuringiensis
Bacillus cereus
Bacillus amyloliquefaciens
Bacillus brevis
Bacillus natto
Bacillus niger
Bacillus aterrimus

Bacillus amylosacchariticus
Bacillus anthracis
Bacillus globigli
Bacillus megaterium
Staphylococcus aureus
Staphylococcus epidermidis
Staphylococcus carnosus
Clostridium acetobutylicum
Pediococcus damnosus
Pediococcus pentosaceus
Pediococcus acidilactlci
Lactobacillus case!
Listeria grayi
Listeria murray!
Listeria monocytogenes
Streptococcus pyogenes
Streptococcus agalactlae
Streptococcus sanguis
Streptococcus salivarlous
Streptococcus cremoris
Streptococcus pneumoniae
Streptococcus avium
Streptococcus faecalis
Streptococcus anginosus
Streptococcus sobrinus
Streptococcus lactis
Streptococcus mutans
Streptococcus equisimilis
Streptococcus thermophylus
Streptococcus miller!
Streptococcus durans
Streptococcus mitior
Streptococcus ferus

Exceptions.

i) Experiments described in Section VI.C.1 which require specific RAC review and IBC approval before initiation of the experiment.

ii) Large-scale experiments (e.g. more than 10 liters of culture) require prior IBC review and approval (see Section VI.C.2.5).

APPENDIX D - PHYSICAL CONTAINMENT

Appendix D-I-Standard Practices and Training

The first principle of containment is a strict adherence to good microbiological practices. Consequently, all personnel directly or indirectly involved in experiments on recombinant DNAs must receive adequate instruction (see Sections VI.D.2 y VI.D.5.5.c). This shall, as a minimum, include 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 research group working with agents with a known or potential biohazard shall have an emergency plan which describes the procedures to be followed if an accident contaminates personnel or the environment. The PI must ensure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan. If a research group is working with a known pathogen for which there is an effective vaccine, the vaccine should be made available to all workers. Where serological monitoring is clearly appropriate, it shall be provided.

Appendix D-II--Physical Containment Levels

The objective of physical containment is to confine organisms containing recombinant DNA molecules and thus to reduce the potential for exposure of the laboratory worker, persons outside of the laboratory, and the environment to organisms containing recombinant DNA molecules. Physical containment is achieved through the use of laboratory practices, containment equipment, and special laboratory design. Emphasis is placed on primary means of physical containment which are provided by 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 in which 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. It should be emphasized that the descriptions and assignments of physical containment detailed below are based on existing approaches to containment of pathogenic organisms. The National Cancer Institute

describes three levels for research on oncogenic viruses which roughly correspond to our BL2, BL3, and BL4.

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 is dependent, however, on the level of biological containment provided by the host-vector system used in the experiment.

Appendix D-II-A--Biosafety Level 1 (BL1)

Appendix D-II-A-1. Standard Microbiological Practices

Appendix D-II-A-1-a. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.

Appendix D-II-A-1-b. Work surfaces are decontaminated once a day and after any spill of viable material.

Appendix D-II-A-1-c. All contaminated liquid or solid wastes are decontaminated before disposal.

Appendix D-II-A-1-d. Mechanical pipetting devices are used; mouth pipetting is prohibited.

Appendix D-II-A-1-e. 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 this purpose only.

Appendix D-II-A-1-f. Persons wash their hands after they handle materials involving organisms containing recombinant DNA molecules and animals and before leaving the laboratory.

Appendix D-II-A-1-g. All procedures are performed carefully to minimize the creation of aerosols.

Appendix D-II-A-1-h. It is recommended that laboratory coats, gowns, or uniforms be worn to prevent contamination or soiling of street clothes.

Appendix D-II-A-2-Special Practices

Appendix D-II-A-2-a. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leakproof container which is closed before being removed from the laboratory.

Appendix D-II-A-2-b. An insect and rodent control program is in effect.

Appendix D-II-A-3. Containment Equipment

Appendix D-II-A-3-a, Special containment equipment is generally not required for manipulation of agents assigned to Biosafety Level 1.

Appendix D-II-A-4. Laboratory Facilities

Appendix D-II-A-4-a. The laboratory is designed so that it can be easily cleaned.

Appendix D-II-A-4-b. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix D-II-A-4-c. Laboratory furniture is sturdy. Spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix D-II-A-4-d. Each laboratory contains a sink for handwashing.

Appendix D-II-A-4-e. If the laboratory has windows that open, they are fitted with fly screens.

Appendix D-II-B--Biosafety Level 2 (BL2)

Appendix D-II-B-1. Standard Microbiological Practices

Appendix D-II-B-1-a. Access to the laboratory is limited or restricted by the laboratory director when work with organisms containing recombinant DNA molecules is in progress.

Appendix D-II-B-1-b. Work surfaces are decontaminated at least once a day and after any spill of viable material.

Appendix D-II-B-1-c. All contaminated liquid or solid wastes are decontaminated before disposal.

Appendix D-II-B-1-d. Mechanical pipetting devices are used; mouth pipetting is prohibited.

Appendix D-II-B-1-e. 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 this purpose only.

Appendix D-II-B-1-f. Persons wash their hands after handling materials involving organisms containing recombinant DNA molecules, and animals, and when they leave the laboratory.

Appendix D-II-B-1-g. All procedures are performed carefully to minimize the creation of aerosols.

Appendix D-II-B-1-h. Experiments of lesser biohazard potential can be carried out concurrently in carefully demarcated areas of the same laboratory.

Appendix D-II-B-2.--Special Practices

Appendix D-II-B-2-a. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leakproof container which is closed before being removed from the laboratory.

Appendix D-II-B-2-b. 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.

Appendix D-II-B-2-c. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific entry requirements (e.g. immunization) enter the laboratory or animal rooms.

Appendix D-II-B-2-d. When the organisms containing recombinant DNA molecules in use in the laboratory require special provisions for entry (e.g. vaccination), a hazard warning sign incorporating the universal biohazard symbol 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 person(s), and indicates the special requirement(s) for entering the laboratory.

Appendix D-II-B-2-e. An insect and rodent control program is in effect.

Appendix D-II-B-2-f. Laboratory coats, gowns, smocks, or uniforms are worn while in the laboratory. Before leaving the laboratory for nonlaboratory areas (e.g., cafeteria, library, administrative offices), this protective clothing is removed and left in the laboratory or covered with a clean coat not used in the laboratory.

Appendix D-II-B-2-g. Animals not involved in the work being performed are not permitted in the laboratory.

Appendix D-II-B-2-h. Special care is taken to avoid skin contamination with organisms containing recombinant DNA molecules; gloves should be worn when handling experimental animals and when skin contact with the agent is unavoidable.

Appendix D-II-B-2-i. All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

Appendix D-II-B-2-j. 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 contain recombinant DNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation 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.

Appendix D-II-B-2-k. Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

Appendix D-II-B-2-1. When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically depending on the agents handled or the function of the facility.

Appendix D-II-B-2-m. 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.

Appendix D-II-B-3--Containment Equipment(*)

Appendix D-II-B-3-a. Biological safety cabinets (Class I or II) or other appropriate personal protective or physical containment devices are used whenever:

Appendix D-II-B-3-a-(1). 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.

(*) Reference: Appendix G-III- Footnotes and References (NIH Guidelines) Fed.Regis.Vol.51 No.88, pp. 16977-78,1986.

Appendix D-II-B-3-a-(2). High concentrations or large volumes of organisms containing recombinant DNA 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.

Appendix D-II-B-4--Laboratory Facilities

Appendix D-II-B-4-a. The laboratory is designed so that it can be easily cleaned.

Appendix D-II-B-4-b. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix D-II-B-4-c. Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix D-II-B-4-d. Each laboratory contains a sink for handwashing.

Appendix D-II-B-4-e. If the laboratory has windows that open, they are fitted with fly screens.

Appendix D-II-B-4-f. An autoclave for decontaminating laboratory wastes is available.

Appendix D-II-C--Biosafety Level 3 (BL3)

Appendix D-II-C-1. Standard Microbiological Practices

Appendix D-II-C-1-a. Work surfaces are decontaminated at least once a day and after any spill of viable material.

Appendix D-II-C-1-b. All contaminated liquid or solid wastes are decontaminated before disposal.

Appendix D-II-C-1-c. Mechanical pipetting devices are used; mouth pipetting is prohibited.

Appendix D-II-C-1-d. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area.

Appendix D-II-C-1-e. Persons wash their hands after handling materials involving organisms containing recombinant DNA molecules, and animals, and when they leave the laboratory.

Appendix D-ll-C-1-f. All procedures are performed carefully to minimize the creation of aerosols.

Appendix D-11-C-1-g. Persons under 16 years of age shall not enter the laboratory.

Appendix *D-II-C-1-h.* If experiments involving other organisms which require lower levels of containment are to be conducted in the same laboratory concurrently with experiments requiring BL3 level physical containment, they shall be conducted in accordance with all BL3 level laboratory practices.

Appendix D-II-C-2-Special Practices

Appendix D-II-C-2-a. Laboratory doors are kept closed when experiments are in progress.

Appendix *D-II-C-2-b. Contaminated materials that are to be* decontaminated at a site away from the laboratory are placed in a durable leakproof container which is closed before being removed from the laboratory.

Appendix D-II-C-2-c. The laboratory director controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

Appendix *D-II-C-2-d.* 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., inmunization), and who comply with all entry and exit procedures enter the laboratory or animal rooms.

Appendix D-II-C-2-e. When organisms containing recombinant DNA 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, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates any special requirements for entering the laboratory, such as the need for immunizations, respirators, or other personal protective measures.

Appendix D-II-C-2-f. All activities involving organisms containing recombinant DNA 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.

Appendix D-II-C-2-g. The work surfaces of biological safety cabinets and other containment equipment are decontaminated when work with organisms containing recombinant DNA molecules is finished.

Plastic-backed paper toweling used on nonperforated work surfaces within biological safety cabinets facilitates clean-up.

Appendix *D-II-C-2-h.* An insect and rodent program is in effect.

Appendix *D-II-C-2-i.* Laboratory clothing that protects street clothing (e.g., solid front or wrap-around gowns, scrub suits, coveralls) is worn in the laboratory. Laboratory clothing is not worn outside the laboratory, and it is decontaminated before being laundered.

Appendix *D-II-C-2-j.* Special care is taken to avoid skin contamination with contaminated materials; gloves should be worn when handling infected animals and when skin contact with infectious materials is unavoidable.

Appendix *D-II-C-2-k.* Molded surgical masks or respirators are worn in rooms containing experimental animals.

Appendix D-II-C-2-l. Animals and plants not related to the work being conducted are not permitted in the laboratory.

Appendix D-II-C-2-m. Laboratory animals held in a BL3 area shall be housed in partial-containment caging systems, such as Horsfall units,(*) 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 in 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.

Appendix D-II-C-2-n. All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

Appendix D-II-C-2-o. Vacuum lines are protected with high efficiency particulate air (HEPA) filters and liquid disinfectant traps.

Appendix D-II-C-2-p. 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 contain

(*) Horsfall, F.L. Jr. and J.H. Baner. Individual Isolation of Infected Animals in a Single Room. J.Bact.40, 569-580,1940.

recombinant DNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation 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.

Appendix D-II-C-2-q. Spills and accidents which result in overt or potential exposures to organisms containing recombinant DNA molecules are immediately reported to the laboratory director. Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained.

Appendix D-II-C-2-r. Baseline serum samples for all laboratory and other at-risk personnel should be collected and stored. Additional serum specimens may be collected periodically depending on the agents handled or the function of the laboratory.

Appendix D-II-C-2-s. 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.

Appendix D-II-C-2-t. Alternative Selection of Containment Equipment. 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 BL3 laboratory using containment equipment specified for the BL4 level of physical containment. Alternative combination of containment safeguards are shown in Table 1.

Appendix D-II-C-3--Containment Equipment(*)

Appendix D-II-C-3-a. Biological safety cabinets (Class I, II or lll) 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 recombinant DNA molecules which pose a threat of aerosol exposure.

(*) Reference: Appendix G-III-Footnotes and References (NIH Guidelines) Fed.Regis.Vol.51 No.88, pp. 16977-78,1986.

These include: manipulation of cultures and of those clinical or environmental materials which may be a source of aerosols; the aerosol challenge of experimental animals; and harvesting infected tissues or fluids from experimental animals and embryonating eggs; and necropsy of experimental animals.

Appendix D-II-C-4--Laboratory Facilities

Appendix D-II-C-4-a. The laboratory is separated from areas which are open to unrestricted foot traffic flow within the building. Passage through two sets of doors is the basic requirement for entry into the laboratory from access corridors 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), airlock, or other access facility which requires passage through two sets of doors before entering the laboratory.

Appendix D-II-C-4-b. 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 decontaminating the area.

Appendix D-II-C-4-c. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix D-II-C-4-d. Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix D-II-C-4-e. Each laboratory contains a sink for hand-washing. The sink is foot, elbow, or automatically operated and is located near the laboratory exit door.

Appendix D-II-C-4-f. Windows in the laboratory are closed and sealed.

Appendix D-II-C-4-g. Access doors to the laboratory or containment module are self-closing.

Appendix D-II-C-4-h. An autoclave for decontaminating laboratory wastes is available preferably within the laboratory.

Appendix D-II-C-4-i. 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 can be discharged to the outside without being filtered or otherwise treated.

Appendix D-II-C-4-j. The HEPA-filtered exhaust air from Class I or Class II biological safety cabinets is discharged directly to the outside or through the building exhaust system. Exhaust air from Class I or II biological safety cabinets may be recirculated within the laboratory if the cabinet is tested and certified at least every twelve 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 is connected to this system in a manner (e.g., thimble unit connection) that avoids any interference with the air balance of the cabinets or building exhaust system.

Appendix D-II-D--Biosafety Level 4 (BL4)

Appendix D-II-D-1. Standard Microbiological Practices

Appendix D-II-D-1-a. Work surfaces are decontaminated at least once a day and immediately after any spill of viable material.

Appendix D-II-D-1-b. Only mechanical pipetting devices are used.

Appendix D-II-D-1-c. Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the laboratory.

Appendix D-II-D-1-d. All procedures are performed carefully to minimize the creation of aerosols.

Appendix D-II-D-2--Special Practices

Appendix D-II-D-2-a. 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 nonbreakable, sealed primary container and then enclosed in a nonbreakable, sealed secondary container which is removed from the facility through a disinfectant dunk tank, fumigation chamber, or an airlock designed for this purpose.

Appendix D-II-D-2-b. No materials, except for biological materials that are to remain in a viable or intact state, are removed from the maximum containment laboratory unless they have been autoclaved or decontaminated before they leave the facility. Equipment or material which might be damaged by high temperatures or steam is decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

AppendixD-II-D-2-c. Only persons whose presence in the facility or individual laboratory rooms are required for program or support purposes are authorized to enter. The supervisor 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 person(s) responsible for the physical security of the facility. Before entering, persons are advised of the potential biohazards and instructed as to appropriate safeguards for ensuring 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.

Appendix D-II-D-2-d. Personnel enter and leave the facility only through the clothing change and shower rooms. Personnel shower each time they leave the facility. Personnel use the airlocks to enter or leave the laboratory only in an emergency.

Appendix D-II-D-2-e. 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.

Appendix D-II-D-2-f. When materials that contain organisms containing recombinant DNA molecules or experimental animals are present in the laboratory or animal rooms, a hazard warning sign incorporating the universal biohazard symbol 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 immunizations or respirators).

Appendix D-II-D-2-g. Supplies and materials needed in the facility are brought in byway of 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.

Appendix D-II-D-2-h. An insect and rodent control program is in effect.

Appendix D-II-D-2-i. Materials (e.g. plants, animals, and clothing) not related to the experiment being conducted are not permitted in the facility.

Appendix D-II-D-2-j. 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 recombinant DNA 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).

Appendix D-II-D-2-k. 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.

Appendix D-II-D-2-1. 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), 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.

Appendix D-II-D-2-m. Alternative Selection of Containment Equipment

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 are shown in Table 1.

Appendix D-II-D-3--Containment Equipment(*)

Appendix D-II-D-3-a. All procedures within the facility with agents assigned to Biosafety level 4 are conducted in the Class III biological safety cabinet or in Class I or II biological safety cabinets used in conjunction with

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(*) Reference: Appendix G-III- Footnotes and References (NIH Guidelines) Fed.Regis.Vol.51 No.88, pp. 16977-78,1986.

one-piece positive pressure personnel suits ventilated by a life-support system.

Appendix *D-II-D-4--Laboratory* Facilities

Appendix *D-II-D-4-a.* 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 personnnel entering and leaving the facility. A double-doored autoclave, fumigation chamber, or ventilated airlock is provided for passage of those materials, supplies, or equipment which are not brought into the facility through the change room.

Appendix *D-II-D-4-b.* Walls, floors, and ceilings of the facility are constructed to form a sealed internal shell which 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 they are connected directly to the liquid waste decontamination system. Sewer and other ventilation lines contain HEPA filters.

Appendix *D-II-D-4-c.* 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.

Appendix *D-II-D-4-d.* Bench tops have seamless surfaces which are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix *D-II-D-4-e.* Laboratory furniture is of simple and sturdy construction, and spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix *D-II-D-4-f.* A foot, elbow, or automatically operated hand-washing sink is provided near the door of each laboratory room in the facility.

Appendix *D-II-D-4-g.* 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 lock. 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.

Appendix *D-II-D-4-h.* 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 backflow-protected distribution system supplying water to the laboratory areas.

Appendix *D-II-D-4-i.* Access doors to the laboratory are self-closing and lockable.

Appendix *D-II-D-4-j.* Any windows are breakage resistant.

Appendix *D-II-D-4-k.* An interlocking 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 only be opened after the autoclave "sterilization" cycle has been completed.

Appendix *D-II-D-4-l.* 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.

Appendix *D-II-D-4-m.* 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 is of demonstrated efficacy against the target or indicator micoorganisms.

Appendix *D-II-D-4-n.* 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.

Appendix *D-II-D-4-o.* 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 taking into consideration prevailing wind direction and velocities. 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 supply air system should air pressures become unbalanced in the laboratory.

Appendix D-II-D-4-p. The treated exhaust air from Class I and II biological safety cabinets can be discharged into the laboratory room environment or the 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, it is connected to this system in a manner (e.g. thimble unit connection) that avoids any interference with the air balance of the cabinets or the facility exhaust air system.

Appendix D-II-D-4-q. 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 airtanks. 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 of 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.

Appendix D-lll - Biological Safety Cabinets

The biological safety cabinets are classified in Class I, Class II and Class III:

Class I:

Is a ventilated cabinet for personnel protection having an inward flow of air away from the operator. The exhaust air from this cabinet is filtered through a high-efficiency particulate air (HEPA) filter. This cabinet is used in

three operational modes: 1) with a full-width open front, 2) with an installed front closure panel (having four 8-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.

Class II:

Is a ventilated cabinet for personnel and product protection having an open front with inward air flow for personnel protection, and HEPA filtered mass recirculated air flow 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.

Class lll.

Is a closed-front ventilated cabinet of gas-tight construction which provides the highest level of personnel protection of all biohazard safety cabinets. The interior of the cabinet is protected from contaminants exterior to the cabinet. The cabinet is fitted with arm-length rubber gloves and is operated under a negative pressure of at least 0.5 inches water gauge. All supply air 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.

Appendix D-IV - Biosafety Level

The biosafety levels are classified in Levels 1(NBL2), 2(NBL3) and 3(NBL4):

Biosafety Level 1: (NBL2)

Is suitable for work involving agents of no known or minimal potential hazard to laboratory personnel and the 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 have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science.

Biosafety Level 2: (NBL3)

Is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that: 1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent 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.

Biosafety Level 3: (NBL4)

Is applicable to clinical, diagnostic, teaching, research or production facilities in which work is done with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents and are supervised by competent scientists who are experienced in working with these agents. All procedures involving the manipulation of infectious material are conducted within biological safety cabinets or other physical containment devices or by personnel wearing 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 Biosafety Level 3 (e.g., access zone, sealed penetration, and directional airflow, etc.). In these circumstances, acceptable safety may be achieved for routine or repetitive operations (e.g., diagnostic procedures involving the propagation of an agent for identification, typing, and susceptibility testing) in laboratories where facility features satisfy Biosafety Level 2 recommendations provided the recommended "Standard Microbiological Practices", "Special Practices" and "Containment Equipment" for Biosafety Level 3 are rigorously followed. The decision to implement this modification of Biosafety Level 3 recommendations should be made only by the laboratory director.

APPENDIX E -BIOLOGICAL CONTAINMENT

Appendix E-I: Levels of Biological Containment

In consideration of biological containment, the vector (plasmid, organelle, or virus) for the recombinant DNA and the host (bacterial, plant, or animal cell) in which the vector is propagated in the laboratory will be considered together. Any combination of vector and host which is to provide 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 nonlaboratory hosts.

The following levels of biological containment (HV, or Host-Vector, systems) for prokaryotes will be established; specific criteria will depend on the organisms to be used.

Appendix E-I-A. HV1.

A host-vector system which provides a moderate level of containment.

Appendix E-I-B. 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 recombinant DNA either via survival of the organisms or via transmission of recombinant DNA to other organisms should be less than $1/10^8$ under specific conditions.

Appendix E-ll. Certification *of* Host-Vector Systems

Appendix E-ll-A. Data to be Submitted for Certification

Appendix E-II-A-1. HV1 Systems Other than E. coli K-12

The following types of data shall be submitted, modified as appropriate for the particular system under consideration: (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 which render this organism less able to

survive or transmit genetic information; (iii) a general description of the range of experiments contemplated with emphasis on the need for developing such an HV1 system.

Appendix E-II-A-2. HV2 Systems.

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 nonpermissive laboratory conditions designed to represent the relevant natural environment; (iv) data or transmissibility of the vector and/or a cloned DNA fragment under both permissive and nonpermissive 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; and (vi) 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.

TABLE 1. POSSIBLE ALTERNATE COMBINATIONS OF PHYSICAL AND BIOLOGICAL CONTAINMENT SAFEGUARDS

CLASSIFICATION OF PHYSICAL AND BIOLOGICAL CONTAINMENT	ALTERNATE PHYSICAL CONTAINMENT			BIOLOGICAL CONTAINMENT
	ALTERNATE LABORATORY FACILITIES	LABORATORY PRACTICES	CONTAINMENT EQUIPMENT	
BL3/HV2	BL3	BL3	BL3	HV2
	BL3	BL3	BL4	HV1
BL3/HV1	BL3	BL3	BL3	HV1
	BL3	BL3	BL2	HV2
BL4/HV1	BL4	BL4	BL4	HV1
	BL4	BL4	BL3	HV2

VII. SUPPLEMENTARY GUIDELINES

REGARDING THE INTRODUCTION OF ORGANISMS AND PRODUCTS ALTERED OR PRODUCED THROUGH GENETIC ENGINEERING WHICH ARE PLANT PESTS, OR ORGANISMS WHICH THERE IS REASON TO BELIEVE ARE PLANT PESTS, AND/OR MAY BE DELETERIOUS TO THE SAFETY OF MAN AND THE ENVIRONMENT

These guidelines are supplemental and are not intended to replace existing sanitary regulations for organisms which are plant pests or for which there is reason to believe are plant pests.

VII.A DEFINITIONS

Terms used in the singular form in this part shall be construed as the plural, and vice versa, as the case may demand. The following terms, when used in this part, shall be construed, respectively, to mean:

VII.A.1 ADMINISTRATOR

The Administrator or Director for Plant Protection and Quarantine, Animal and/or Plant Health Inspection Service, Ministry or Department of Agriculture, or any other officer or employee of the Ministry or Department to whom authority to act in his/her stead has been or may hereafter be delegated.

VII.A.2 DONOR ORGANISM

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

VII.A.3 ENVIRONMENT

All the land, air, and water; and all living organisms in association with land, air and water, or other organisms.

VII.A.4 GENETIC ENGINEERING

The genetic modification of organisms by recombinant DNA techniques.

VII.A.5 INSPECTOR

Any employee of Plant Protection and Quarantine and/or Plant Health Inspection Service, Ministry or Department of Agriculture, or other person,

authorized by the Administrator or Director in accordance with the law to enforce the provisions of this part.

VII.A.6 INTRODUCE OR INTRODUCTION

To move into or through the country, to release into the environment, to move interstate, or any attempt thereat.

VII.A.7 MOVE (MOVING, MOVEMENT)

To ship, offer for shipment, offer for entry, import, receive for transportation, carry, or otherwise transport or move, or allow to be moved into, through, or within the country.

VII.A.8 ORGANISM

Any active, infective, or dormant stage or life form of an entity characterized as living, including vertebrate and invertebrate animals, plants, bacteria, fungi, mycoplasms, mycoplasm-like organisms, as well as entities such as viroids, viruses, or any entity characterized as living, related to the foregoing.

VII.A.9 PERMIT

A written permit issued by the Administrator or Director for the introduction of a regulated article under conditions determined by the Administrator or Director not to present a risk of plant pest introduction.

VII.A.10 PERSON

Any individual, partnership, corporation, company, society, association, or other organized group, national or international independent of diplomatic immunity.

VII.A.11 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 thereof, capable of propagation (e.g. pollen, seeds, cells, tubers, stems).

(*) The taxonomic scheme for the plant kingdom is that found in Synopsis and Classification of Living Organisms by S.P. Parker, McGraw Hill (1984).

VII.A.12 PLANT PEST

Any living stage (including active and dormant forms) of insects, mites, nematodes, slugs, snails, protozoa, or other invertebrate animals, bacteria, fungi, other parasitic plants or reproductive parts thereof; viruses; or any organisms similar to or allied with any of the foregoing or any infectious agents or substances, which can directly or indirectly injure or cause disease or damage in or to any plants or parts thereof, or any processed, manufactured, or other products of plants.

VII.A.13 PLANT PROTECTION AND QUARANTINE

The organizational unit within the Ministry or Department of Agriculture, delegated responsibility for enforcing provisions of the Plant Protection and Quarantine Law, and related legislation, and quarantine and regulations promulgated thereunder.

VII.A.14 PRODUCT

Anything made by or from, or derived from an organism, living or dead.

VII.A.15 RECIPIENT ORGANISM

The organism which receives genetic material from a donor organism.

VII.A.16 REGULATED ARTICLE

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 Section VII.B of this part and meets the definition of plant pest, 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 Administrator or Director determines is a plant pest or has reason to believe is a plant pest. Excluded are recipient microorganisms which are not plant pests and which have resulted from the addition of genetic material from a donor organism where the material is well characterized and contains only non-coding regulatory regions.

VII.A.17 RELEASE INTO THE ENVIRONMENT

The use of a regulated article outside the constraints of physical confinement that are found in a laboratory, contained greenhouse, or a fermenter or other physically contained structure appropriate to the regulated article.

VII.A.18 RESPONSIBLE PERSON

The person who has authority and will maintain control over the introduction of the regulated article and assure that all conditions contained in the permit and requirements in this part are complied with.

VII.A.19 MINISTER OR SECRETARY

The Minister or Secretary of Agriculture, or any other officer or employee of the Ministry or Department of Agriculture to whom authority to act in his/her stead has been or may hereafter be delegated.

VII.A.20 VECTOR OR VECTOR AGENT

Organisms or objects used to transfer genetic materials or the cellular components from the donor organism to the recipient organism.

VII.B GROUPS OF ORGANISMS WHICH ARE OR CONTAIN PLANT PESTS

The organisms that are or contain plant pests are included in the taxa or group of organisms contained in the following list. Within any taxonomic series included on the list, the lowest unit of classification actually listed is the taxon or group which may contain organisms which are regulated. Organisms belonging to all lower taxa contained within the group listed are included as organisms that may be or may contain plant pests, and are regulated if they meet the definition of plant pest in Section VII.A.12(*).

Any genetically engineered organism composed of DNA or RNA sequences, organelles, plasmids, parts, copies, and/or analogs, of or from any of the groups of organisms listed below shall be deemed a regulated article if it also meets the definition of plant pest in Section VII.A.12.

(*) **This is not an exhaustive list, but may be modified to meet the needs of any particular country. Any organism belonging to any taxa contained within any listed genera or taxa is only considered to be a plant pest if the organism "can directly or indirectly injure, or cause disease, or damage in any plants or parts thereof, or any processed, manufactured, or other products of plants. Thus, a particular unlisted species within a listed genus would be deemed a plant pestfor purposes mentioned in this Section if the scientific literature refers to the organism as a cause of direct or indirect injury, disease, or damage to any plants, plant parts or products of plants.**

GROUP

Viroids

Prokaryotae Superkingdom

Kingdom Virus

All members of groups containing plant viruses, and all other plant and insect viruses.

Kingdom Monera

Division Bacteria

Family Pseudomonadaceae

Genus Pseudomonas

Genus Xanthomonas

Family Rhizobiaceae

Genus Rhizobium

Genus Bradyrhizobium

Genus Agrobacterium

Genus Phyllobacterium

Family Enterobacteriaceae

Genus Ertivinia

Family Streptomycetaceae

Genus Stretomyces

Family Actinomycetaceae

Genus Actinomyces

Coryneform Group

Genus Clavibacter

Genus Arthrobacter

Genus Curtobacterium

Genus Corynebacteria

Gram-negative phleom-limited bacteria associated with plant diseases

Gram-negative xylem-limited bacteria associated with plant diseases

And all other bacteria associated with plant or insect diseases

Rickettsiaceae

Rickettsial-like organisms associated with insect diseases

Class Mollicutes

Order Mycoplasmatales

Family Spiroplasmataceae

Genus Spiroplasma

Mycoplasma-like organisms associated with plant diseases

Mycoplasma-like organisms associated with insect diseases
Superkingdom *Eukaryotae*
Kingdom Plantae
Subkingdom *Thallobionta*
Division *Chlorophyta*
Genus Cephaleuros
Genus *Rhodochytrium*
Genus *Phyllosiphon*
Division Myxomycota
Class Plasmodiophoromycetes
Division Eumycota
Class Chytridiomycetes
Order Chytridiales
Class Oomycetes
Order Lagenidiales
Family Lagenidiaceae
Family Olpidiopsidaceae
Order Peronosporales
Family Albuginaceae
Family Peronosporaceae
Family Pythiaceae
Order Saprolegniales
Family Saprolegniaceae
Family Leptolegniellaceae
Class Zygomycetes
Order Mucorales
Family Choanephoraceae
Family Mucoraceae
Family *Entomophthoraceae*
Class Hemiascomycetes
Family Protomycetaceae
Family Taphrinaceae
Class Loculoascomycetes
Order Myriangiales
Family Elsinoeaceae
Family Myriangiaceae
Order Asterinales
Order *Dothideales*

Order Chaetothyriales
Order Hysteriales
Family Parmularlaceae
Family Phillipsiellaceae
Family Hysteriaceae
Order Pleosporales
Order Melanommatales
Class Plectomycetes
Order Eurotiales
Family Ophiostomataceae
Order Ascophaerales
Class Pyrenomycetes
Order Erysiphales
Order Mellolales
Order Xylariales
Order Diaporthales
Order Hypocreales
Order Clavicipitales
Class Discomycetes
Order Phacidiales
Order Helotiales
Family Ascocorticiceae
Family Hemiphacidiaceae
Family Dermataceae
Family Sclerotiniaceae
Order Cytarriales
Order Medeolariales
Order Pezziales
Family Sarcosomataceae
Family Sarcocyphaceae
Class Teliomycetes
Class Phragmobasidiomycetes
Family Auriculariaceae
Family Ceratobasidiaceae
Class Hymenomycetes
Order Exobasidiales
Order Agaricales
Family Corticiaceae

Family Ymenochaetaceae
Family Echinodontiaceae
Family Fistulinaceae
Family Clavariaceae
Family Polyporaceae
Family Tricholomataceae
Class Hyphomycetes
Class Coelomycetes
And all other fungi associated with plant or insect diseases
Subkingdom Embryobionta
Division Magnoliophyta
Family Balanophoraceae--parasitic species
Family Cuscutaceae--parasitic species
Family Hydnoraceae--parasitic species
Family Krameriaceae--parasitic species
Family Lauraceae--parasitic species
Genus Cassytha
Family Lennoaceae--parasitic species
Family Loranthaceae--parasitic species
Family Myzodendraceae--parasitic species
Family Olacaceae--parasitic species
Family Oroganchaceae--parasitic species
Family Rafflesiaceae-parasitic species
Family Santalaceae--parasitic species
Family Scrophulariaceae--paraistic species
Genus Alectra
Genus Barsia
Genus Buchnera
Genus Buttonia
Genus Castilleja
Genus Centranthera
Genus Cordylanthus
Genus Dasistoma
Genus Euphrasis
Genus Gerardia
Genus Harveya
Genus Hyobanche
Genus Lathraea

Genus Melampyrum
Genus Melasma
Genus *Orthantha*
Genus Orthocarpus
Genus Pedicularis
Genus *Rhamphicarpa*
Genus *Rhinanthus*
Genus *Schwalbea*
Genus Seymeria
Genus Siphonostegia
Genus Sopubia
Genus Striga
Genus Tozzia
Family Viscacese--parasitic species
Kingdom Animalia
Subkingdom Protozoa
Genus Phytomonas
And all Protozoa associated with insect diseases
Subkingdom Eumetazoa
Phylum Nemata
Class *Secernentea*
Order *Tylenchida*
Family Anguinidae
Family *Belonolaimidae*
Family Caloosiidae
Family Criconemaddae
Family *Dolichodoridae*
Family *Fergusobiidae*
Family Hemicycliophoridae
Family Heteroderidae
Family Hoplolalmidae
Family Meloidogynidae
Family *Nacobbidae*
Family Neotylenchidae
Family *Nothotylenchidae*
Family Paratylenchidae
Family *Pratyenchidae*
Family *Tylenchidae*

Family Tylenchulidae
Order Aphelenchida
Family Aphelenchoididae
Class Adenophorea
Order Dorylaimida
Family Longidoridae
Family Trichodoridae
Phylum Mollusca
Class Gastropoda
Subclass Pulmonata
Order Basommatophora
Superfamily Planorbacea
Order Stylommatophora
Subfamily Strophocheilacea
Family Succineidae
Superfamily Achatinacae
Superfamily Arionacae
Superfamily Limacacea
Superfamily Helicacea
Order Systellommatophora
Superfamily Veronicellacea
Phylum Arthropoda
Class Arachnida
Order Parasitiformes
Suborder Mesostigmata
Superfamily Ascoidea
Superfamily Dermanyssoidea
Order Acariformes
Suborder Prostigmata
Superfamily Eriophyoidea
Superfamily Tetranychoidea
Superfamily Eupodoidea
Superfamily Tydeoidea
Superfamily Erythraenoidea
Superfamily Trombidioidea
Superfamily Hydryphantoidea
Superfamily Tarsonemoidea
Superfamily Pyemotoidea

Suborder Astigmata
Superfamily Hemisarcoptoidea
Superfamily Acaroidea
Class Diplopoda
Order Polydesmida
Class Insecta
Order Collembola
Family Sminthoridae
Order Isoptera
Order Thysanoptera
Order Orthoptera
Family Acrididae
Family Gryllidae
Family Gryllacrididae
Family Gryllotalpidae
Family Phasmatidae
Family Ronaleidae
Family Tettigoniidae
Family Tetrigidae
Order Hemiptera
Family Thaumastocoridae
FamilyAradidae
Superfamily Piesmatoidea
Superfamily Lygaeoidea
Superfamily Idiostoloidea
Superfamily Coreoidea
Superfamily Pentatomoidea
Superfamily Pyrrhocoroidea
Superfamily Tingoidea
Superfamily Miroidea
Order Homoptera
Order Coleoptera
Family Anobiidae
Family Apionidae
Family Anthribidae
Family Bostrichidae
Family Brentidae
Family Bruchidae

Family Buprestidae
Family Byturidae
Family Canthaddae
Family Carebidae
Family Cerambycidae
Family Chrysomelidae
Family Coccinellidae
    Subfamily Epilachninae
Family Curculionidae
Family Dermestidae
Family Elateridae
Family Hydrophilidae
    Genus Helophorus
Family Lyctidae
Family Meloldae
Family Mordellidae
Family Platypodidae
Family Scarabaeidae
    Subfamily Melolonthinae
    Subfamily Rutelinae
    Subfamily Cetoniinae
    Subfamily Dynastinae
Family Scolytidae
Family Selbytidae
Family Tenebrionidae
Order Lepideoptera
Order Díptera
Family Agromyzidae
Family Anthomyiidae
Family Cecidomyfidae
Family Chloropidae
Family Ephydridae
Family Lonchaeidae
Family Muscidae
    Genus Atherigona
Family Otitidae
    Genus Euxeta
Family Syrphidae

Family Tephritidae
Family Tipulidae
Order Hymenoptera
Family Apidae
Family Caphidae
Family Chalcidae
Family Cynipidae
Family Eurytomidae
Family Formicidae
Family Psilidae
Family Siricidae
Family Tenthredinidae
Family Torymidae
Family Xylocopidae

Unclassified organisms and/or organisms whose classification is unknown.

VII.C PERMITS FOR THE INTRODUCTION OF A REGULATED ARTICLE

VII.C.1 Application for Permit. A written application for a permit to introduce a regulated article shall be submitted by the responsible person on an application form obtained from Plant Protection and Quarantine of the Department of Agriculture (in some countries it will be the Biotechnology Units or Programs which are on a higher level than the Ministries or Departments of Agriculture). If there are portions of the application deemed to contain trade secrets or confidential business information (CBI), each page of the application containing such information should be marked "CBI Copy". In addition, those portions of the application which are deemed "CBI" shall be so designated.

VII.C.2 Permit for Release into the Environment. An application for the release into the environment of a regulated article shall be submitted at least 120 days in advance of the proposed release into the environment. An initial review shall be completed by Plant Protection and Quarantine within 30 days of the receipt of the application. If the application is complete, the responsible individual shall be notified of the date of receipt of the application for purposes of advising the applicant when the 120 days review period commenced.(*) If the application is not complete, the

(*) **The 120 days review period would be extended if preparation of an environment impact statement in addition to an environmental assessment was necessary.**

responsible individual will be advised what additional information must be submitted. Plant Protection and Quarantine shall commence the 120-day review period upon receipt of the additional information, assuming the additional information submitted is adequate. When it is determined that an application is complete, Plant Protection and Quarantine shall submit to the Department of Agriculture, of the political-administrative region where the release is planned, a copy of the initial review and a copy of the application marked, "CBI Deleted", or "No CBI" for notification and review. The application shall Include the following information:

VII.C.2.1 Name, title, address, telephone number, signature of the responsible person and type of permit requested (for introduction, interstate movement, or release into the environment);

VII.C.2.2 All scientific, common, and trade names, and all designations necessary to identify the: Donor organism(s); recipient organism(s); vector or vector agent(s); constituent of each regulated article which is a product; and, regulated article;

VII.C.2.3 Names, addresses, and telephone numbers of the persons who developed and/or supplied the regulated article;

VII.C.2.4 A description of the means of transport (e.g., mail, common carrier, baggage, or handcarried (and by whom));

VII.C.2.5 A description of the anticipated or actual expression of the altered genetic material in the regulated article and how that expression differs from the expression in the non-modified parental organism (e.g., morphological or structural characteristics, physiological activities and processes, number of copies of inserted genetic material and the physical state of this material inside the recipient organism (integrated or extrachromosomal, products and secretions, growth characteristics);

VII.C.2.6 A detailed description of the molecular biology of the system (e.g., donor-recipient-vector) which was/will be used to produce the regulated article;

VII.C.2.7 Country and locality where the donor organism, recipient organism, vector or vector agent, and regulated article were collected, developed, and produced;

VII.C.2.8 A detailed description of the purpose for the introduction of the regulated article including a detailed description of the proposed experimental and/or production design;

VII.C.2.9 The quantity of the regulated article to be introduced and proposed schedule and number of introductions;

VII.C.2.10 A detailed description of the processes, procedures, and safeguards which have been used or will be used in the country of origin and in the recipient country to prevent contamination, release, and dissemination in the production of the: donor organism; recipient organism; vector or vector agent; constituent of each regulated article which is a product; and regulated article;

VII.C.2.11 A detailed description of the intended destination (including final and all intermediate destinations), uses, and/or distribution of the regulated article (e.g., greenhouses, laboratory, or growth chamber location; field trial location; pilot project location; production, propagation, and manufacture location; proposed sale and distribution location);

VII.C.2.12 A detailed description of the proposed procedures, processes, and safeguards which will be used to prevent escape and dissemination of the regulated article at each of the intended destinations;

VII.C.2.13 A detailed description of any biological material (e.g., culture medium, or host material) accompanying the regulated article during transport; and

VII.C.2.14 A detailed description of the proposed method of safe final disposal of the regulated article.

VII.C.2.15 Contingency Plan for clean up and decontamination in case of accidental spill.

VII.C.3 Premises Inspection. An inspector may inspect the site or facility where regulated articles are proposed, pursuant to a permit, to be released into the environment or contained after their interstate movement or importation.

VII.C.4 Permit Conditions. A person who is issued a permit and his/her employees or agents shall comply with the following conditions, and any supplemental conditions which shall be listed on the permit, as deemed by the Administrator or Director to be necessary to prevent the dissemination and establishment of plant pests;

VII.C.4.1 The regulated article shall be maintained and disposed of (when necessary) in a manner so as to prevent the dissemination and establishment of plant pests.

VII.C.4.2 All packing material, shipping containers, and any other material accompanying the regulated article shall be treated or disposed of in such a manner so as to prevent the dissemination and establishment of plant pests.

VII.C.4.3 The regulated article shall be kept separate from other organisms, except as specifically allowed in the permit;

VII.C.4.4 The regulated article shall be maintained only in areas and premises specified in the permit;

VII.C.4.5 An inspector shall be allowed access, during regular business hours, to the place where the regulated article is located and to any records relating to the introduction of a regulated article;

VII.C.4.6 The regulated article shall, when possible, be kept identified with a label showing the name of the regulated article, and the date of importation;

VII.C.4.7 The regulated article shall be subject to the application of measures determined by the Administrator or Director to be necessary to prevent the accidental or unauthorized release of the regulated article;

VII.C.4.8 The regulated article shall be subject to the application of remedial measures (including disposal) determined by the Administrator or Director to be necessary to prevent the spread of plant pests;

VII.C.4.9 A person who has been issued a permit shall submit to Plant Protection and Quarantine monitoring reports on the performance characteristics of the regulated article, in accordance with any monitoring reporting requirements that may be specified in a permit;

VIII. GENERAL REQUIREMENTS FOR NEW DRUGS AND BIOLOGICS FOR HUMAN USE

A new drug is, in general terms, a drug not generally recognized by qualified scientific experts as safe and effective for the proposed use. New drugs may not be marketed unless they have been approved as safe and effective for their intended uses. Clinical investigations on human subjects by qualified experts are a prerequisite for the determination of safety and effectiveness. Sponsors of investigations of new drugs or new uses of approved drugs should file a petition to the Ministries of Health authorities to conduct clinical investigations on human subjects. The petition must contain information to demonstrate the safety of proceeding to test the drug in human subjects, including, for example, drug composition, manufacturing and control data, results of animal testing, training and experience of investigators, and a plan for clinical investigation. In addition, assurance of informed consent and protection of the rights and safety of human subjects is required.

VIII.A APPROVAL OF APPLICATIONS

Approval of a New Drug Application or an abbreviated NDA by the health authorities is required before the new drug can be marketed. The NDA must contain, among other information, the following:

VIII.A.1 A list of components of the drug and a statement of the composition of the drug product;

VIII.A.2 A description of the manufacturing and packaging procedures and controls for the drug product;

VIII.A.3 A description of the nonclinical studies concerning the drug's pharmacological actions and toxicological effects;

VIII.A.4 A description and analysis of each clinical study; and

VIII.A.5 A description and analysis of any other data or information relevant to an evaluation of the safety and effectiveness of the drug product, including commercial marketing experience.

NDA holders who intend to market an approved drug under conditions other than those approved in the NDA must submit a supplemental NDA containing clinical evidence of the drug's safety and effectiveness for the added indications. Extensive changes such as a change in formula,

manufacturing process, or method of testing differing from the conditions of approval outlined in the NDA may also require additional clinical testing.

Biological products must also be approved by the corresponding authorities of Ministries of Public Health. A biological product is "any virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, or analogous product, applicable to the prevention, treatment, or cure of diseases or injuries of man". Unapproved biological products are regulated under the same regulation as new drugs during the NDA phase. Prior to marketing, separate licenses are issued for the manufacturing establishment and the biological product. The manufacturing establishment and the biological product must meet standards (including any country standards specific for the product) designed to ensure the safety, purity, potency, and efficacy of the product. To obtain a license, the facility must also pass a prelicensing inspection. Licensed products are subject to specific requirements for lot release in each country.

Manufacturers of new drugs and biologics must operate in conformance with current good manufacturing practice (CGMP) regulations. These regulations require adequately equipped manufacturing facilities, adequately trained personnel, stringent control over the manufacturing process, and appropriate finished product examination. CGMP's are designed to protect the integrity and purity of the product.

The sponsor's process techniques are also considered in the health authorities reviews and communications for the development of appropriate information on which the submission of an NDA or biological product license application would be based. For example, the use of recombinant DNA technology to manufacture new drugs or biological products may result in products that differ from similar products manufactured with conventional methods. Determination of the extent of testing required will depend upon the nature of the particular product. In some instances the molecular structure of the product may differ from the structure of the active molecule in nature. For example, the first human growth hormone manufactured using recombinant microorganism has an extra amino acid, an amino-terminal methionine; hence, it is an analogue of the native hormone. Such differences could affect the drugs's activity or immunogenicity and, consequently, could affect the extent of testing required.

Another consideration in the review of new drugs or biological products produced by DNA recombinant techniques is whether the manufacturing process includes adequate quality controls. For example,

the occurrence of mutations in the coding sequence of the cloned gene during fermentation could give rise to a subpopulation of molecules with an anomalous primary structure and altered activity. This is a potential problem inherent in the production of polypeptides in any fermentation process. As with conventionally produced products, assurance of adequate processing techniques and controls is important in the manufacturing of any biotechnology-produced new drug or biological product. Review of the production of human viral vaccines routinely involves a number of considerations including the purity of the media and the serum used to grow the cell substrate, the nature of the cell substrate, and the characterization of the virus. In the case of live viral vaccine, the final product is biologically active and is intended to replicate in the recipient. Therefore, the composition, concentration, subtype, immunogenicity, reactivity, and non pathogenicity of the vaccine preparation are all considerations in the final review, whatever the techniques employed in "engineering" the virus. However, special considerations may arise based upon the specific technology employed. For example, a hepatitis B vaccine produced in yeast (via recombinant DNA techniques) would be monitored for yeast cell contaminants, while distinctly different contaminants would be of concern in a similar vaccine produced from the plasma of infected patients.

The proposed plan for the authorization of new drugs is based on procedures used in several countries and approved by WHO, therefore it is desired that each country establish a system based on these recommendations.

Nucleic acids or viruses used for human gene therapy will be subject to the same requirements as other biological drugs.

To provide guidance to current or prospective manufacturers of drugs and biological products, the authorities should elaborate and distribute a series of documents describing points that manufacturers might wish to consider in the production and testing of products. These documents similar to "Points to Consider" from the FDA/USA should include several topics: interferon, monoclonal antibodies, products of recombinant DNA technology, and the use of new cell substrates.

IX. GENERAL REQUIREMENTS FOR ANIMAL FOOD ADDITIVES AND DRUGS

Animal food additives and drugs are subject to similar mandatory requirements as the like products for use in humans. Drugs, food additives and biologic products for animals, however, are generally licensed by the Veterinary Services of the Ministries or Secretaries of Agriculture/Health.

New animal drugs and Animal Drug Application (NADA) must go through a procedure similar to that required for human drugs, as discussed earlier. However, in general, regulations do not require advance approval of the authorities for clinical investigations for the drug, although authorization is required for use of edible products derived from food-producing animals in which the drug has been used. The data must be specific for each animal species for which the drug is intended. For NADA approval, it must be shown that the product is safe and effective when used in accordance with approved label directions. Also, it must be shown that those drugs which are intended for use in food-producing animals and used in accordance with approved label directions, do not accumulate as unsafe residues in the edible tissues of the animal at the time of slaughter. Moreover, the manufacturer must submit acceptable methods for measurement of any drug residue in edible tissues. Further, animal drugs, including premixes for use in medicated feeds must be manufactured in conformance with CGMPs. Substances that are used in animal feeds, other than drugs, and that are produced by biotechnology, are considered to be food additives and require approval of a separate food additive petition (FAP), even though a similar substance is currently approved as a food additive.

There have been questions about the requirement of an original application for a biotechnology product, even when the product is identical to a currently approved animal drug held by the same applicant. In the U.S. the FDA's Center for Veterinary Medicine (CVM) has determined that, when the new substance produced by biotechnology is identical or virtually identical to an approved substance produced by conventional technology, only a supplemental application is necessary. Of course, in this instance the sponsor of the biotechnology product must also be the sponsor of the conventionally produced product. If, on the other hand, the new substance produced by biotechnology is significantly different from that produced by conventional means, an original application will be needed.

Two examples, each involving the adoption of rDNA technology as an alternative means of producing a substance that is currently the subject of

an approved NADA, will illustrate. In the first example, the drug is (or appears to be) unchanged by the new production method. Under the current regulations, such a departure in manufacturing procedure requires a supplemental application which requires approval before implementation. The supplement would be a Category II supplement under CVM's supplemental policy in that it involves a revised method of synthesis or fermentation for the new drug substance. However, in accordance with the CMV's supplemental policy the underlying safety and effectiveness data supporting the original NADA usually would not be reviewed (for compliance with contemporary standards) since there is likely no increased risk of human exposure to the drug. Data may be required to demonstrate the new animal drug product is essentially biologically equivalent to the drug product for which approval has already been granted *and* does not contain hazardous impurities arising from the change in *manufacturing procedure*.

In the second example, a new method of manufacture changes the molecular structure or chemical composition of the active ingredient. Such a change in the identity of the new animal drug normally will require an original new animal drug application and subsequent publication of a notice of approval. Ordinarily, an original NADA requires complete safety and effectiveness studies, meeting contemporary standards.

It may be possible to regard the new application as if it were a Category II supplement. This finding would be dependent upon data showing the new substance to be sufficiently similar to the original in terms of its pharmacology, toxicology, bioequivalence, and metabolism.

Thus, regardless of the type of application required, there is no legal requirement for the generation of new safety and effectiveness data if the applicant has access to previously submitted data, and there is no scientific need as judged by the regulatory agency.

X. GENERAL REQUIREMENTS FOR MEDICAL DEVICES

Medical devices for human use are generally included in the corresponding sanitary regulations, as equipment and medical aids for diagnosis and treatment. In general, a device is a health care product that does not achieve any of its principal intended purposes by chemical action in or on the body or by being metabolized. Devices include diagnostic aids such as reagents, antibiotic sensitivity discs, and test kits for in vitro diagnosis of disease. These products should follow the established procedures for diagnostic products in general.

XI. GENERAL REQUIREMENTS FOR FOODS

Generally in the sanitary laws and regulations of the countries for the safety of food, no particular statutory provision or regulation deals expressly with food produced by the new rDNA biotechnology. Accordingly, when confronted by an issue concerning the regulation of food produced by this new biotechnology, the relevant statutory or regulatory provisions presently established dealing with the safety of this food produced with conventional technologies will apply.

Normally, the U.S. law established, in part, that a food is adulterated if it bears or contains any poisonous or deleterious "added substance which may render it injurious to health". Courts have agreed with the authorities' interpretation of this section that any substance that is not an inherent constituent of food may be regulated as an "added substance". Furthermore, if the quantity of the constituent exceeds the amount that would normally be present because of some technological adjustment to the product, that excess quantity may also be viewed as "added substance" within the meaning of the law. Thus, the law applies to most of the harmful substances that may occur in human food. For example, if a food produced by new technology contains a higher level of a substance than it might ordinarily have, then that level "may be injurious to health" and the sanitary authorities should regulate the product. Similarly, if a food produced by new biotechnology contains, as a result of the production process, a harmful or deleterious substances not contained ordinarily in the food, the food could be in violation of the law.

The other primary statutory provisions relied upon to determine the safety of food and food constituents are the provisions on food additives normally included in the law. The definition of food additive includes both artificial and natural substances. The definition provides that: the term food additive means any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food (including any substance intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food; including any source of radiation intended for any such use), if such substance is not generally recognized as safe by qualified experts.

If the substance is Generally Recognized As Safe (GRAS) for a given food use, the product is not a food additive.

Comments questioned whether a substance (including microbes) that is GRAS could lose its GRAS status solely because it was produced or modified by new technology. The answer is yes, if the substance (and its contaminants) has been altered in such a way that it can no longer be generally recognized by qualified experts to be safe. In this instance, the substance would be a food additive and the corresponding provisions would apply. In order to be lawfully used in food, a food additive must be the subject of an approved food additive regulation, published upon approval of a food additive petition. The health authorities may not approve a food additive regulation until certain basic evidentiary criteria are met. Most important of these is that the additive must be shown to be safe under the conditions that it will be used. This requires a demonstration to a reasonable certainty that the additive will not adversely affect the health of consumers.

The future anticipates that the *techniques* of new biotechnology used in producing food will, for the most part, involve rDNA and microbial isolation. Certain general principles are recommended that would be followed to determine the safety of foods produced by such techniques.

When determining the safety of food produced by rDNA techniques, the agency takes into consideration, but is not restricted to, whether:

XI.A.1 The cloned DNA as well as the vector used are properly identified;

XI.A.2 The details of the construction of the production organisms are available;

XI.A.3 There is information documenting that the inserted DNA is well-characterized(*) and free from sequences that code for harmful products, and

XI.A.4 The food produced is purified,(**) characterized, and standardized.

When determining the safety of food produced by microbial isolation, the authorities will take into consideration, but are not restricted to, whether:

(*) **"Well characterized" means that the producer can document the exact nucleotide sequence of the insert and any flanking nucleotides.**

(**) **"Purified" means to achieve a level of purity satisfactory for the intended uses of the product.**

XI.B.1 The microbial isolate used for production is identified taxonomically, and if the strain of the isolate has been genetically manipulated; whether each strain contributing genetic information to the production strain is identified;

XI.B.2 The cultural purity and genetic stability of isolate has been maintained;

XI.B.3 Fermentation has been performed with a pure culture and monitored for purity;

XI.B.4 The microbial isolate used for production also produces antibiotics or toxins;

XI.B.5 The isolates are pathogenic:(*)

XI.B.6 Viable cells of the production strain are present in the final product.

As a general rule, the extent of testing required on a food product produced by rDNA technology will depend upon many factors, including the novelty of the substances used to produce the food, the purity of the resulting product, and the estimated consumption of the product.

(*) A pathogen is a virus or microorganism (including its viruses and plasmids, if any) that has the ability to cause disease in other living organisms (i.e., humans, animals, plants, microorganisms). A microorganism will be included within this definition if: 1.a The microorganism belongs to a pathogenic species, according to sources identified by the authorities, or from information known to the producer that the organism is a pathogen; excepted are organisms belonging to a strain used for laboratory research or commercial purposes and generally recognized as nonpathogenic according to sources identified by the authorities, or information known to the producer and the appropriate authorities; an example of a nonpathogenic strain of species which contains a pathogenic strain is Escherichia coli K-12; examples of non-pathogenic species are Bacillus subtilis, Lactobacillus acidophilus, and Saccharomyges species. 1.b The microorganism has been derived from a pathogen or has been deliberately engineered such that it contains genetic material from a pathogenic organism as defined in item 1.a above. Excepted are genetically engineered organisms developed by transferring a well-characterized, non-coding regulatory region from a pathogenic donor to a non-pathogenic recipient. "Well-characterized, non-coding regulatory region" means that the producer of the microorganism can document the following: 2.a The exact nucleotide base sequence of the regulatory region and any inserted flanking nucleotides; 2.b The regulatory region and any inserted flanking nucleotides do not code independently for protein, peptide, or functional RNA molecules; and, 2.c The regulatory region solely controls the activity of other sequences that code for protein or peptide molecules or act as recognition sites for the initiation of nucleic acid or protein synthesis.

This definition excludes organisms such as competitors or colonizers of the same substrates, commensal or mutualistic microorganisms, or opportunistic pathogens.

XII. POINTS TO CONSIDER IN PREPARING PROPOSALS FOR RESEARCH INVOLVING RECOMBINANT DNA TECHNOLOGY

In Section VI.C.1 the points which need to be considered in preparing all proposals for evaluation by the NBTAC (RAC) Committee and the IBC are listed. In Section VI.C.2 the experiments that require approval of the IBC are listed. In Section VI.C.3 the experiments that require only notification to the IBC when initiated are described.

The depth and detail of the evaluation will vary according to the known characteristics of the species being released, the nature of the inserted genetic traits and the type of ecosystem into which it is planned to make the release. For example, more information would be desirable and the assessment more detailed if the parent or modified organism is pathogenic or toxic to humans, plants or animals; has a function which could be ecologically disruptive; has a poorly characterised genome or if the genetic material is unstable or could be readily transmitted to other organisms.

The NBTAC assumes that organizations will conduct experimental field trials before proposing the general release or sale of products incorporating (recombinant DNA) modified organisms. Consequently, the points listed below are oriented towards experimental trials. If, however, an organization intends to proceed directly to a general release then the information provided in response to the points should reflect the circumstances of the intended use or release (e.g., controlling access may not be relevant but determining the impact on non-target species may be more complex than for a release in a single location).

XII.A **CONSIDERATIONS COMMON TO ALL PROPOSALS**

XII.A.1 What is the aim of the proposed research? What are the benefits of this approach compared to other available methods?

XII.A.2 What organism is to be released? What is the genetic modification and what change is it expected to make to the phenotype of the

XII.B REQUIREMENTS

These Guidelines require researchers wishing to use the recombinant DNA technique to submit a proposal giving details of their project to the Institutional Biosafety Committee (IBC) responsible for the facilities where the work is to be performed. Where the work is to be carried out in more

than one organization both IBC's must be informed. The IBC must send a copy of each proposal to the NBTAC either for information or for a recommendation on the conditions under which the work should be carried out.

Researchers are expected to follow all the requirements of these Guidelines including adherence to the specified combinations of physical and biological containment approved by their IBC for the project.

XII.C PROPOSAL FORMS

Copies of both the proposal form and the form to be completed if whole plants are to be used in the work are included in Appendix F. A copy of the form to be completed by the IBC for its assessment of proposals .s also included.

All correspondence with the NBTAC should, at least initially be directed through the IBC.

XII.D PROCEDURES TO BE FOLLOWED FOR APPROVAL OF PROPOSALS TO DO RECOMBINANT DNA RESEARCH

This Section summarizes the procedures to be followed by researchers and IBCs regarding proposals to do recombinant DNA work:

XII.D.1 Research and Development Investigator

An investigator proposing to do recombinant DNA work must:

XII.D.1.1 Examine the categories of work detailed in the form to determine whether or not the proposed work comes within the scope of these Guidelines and if so, within which category;

XII.D.1.2 If the work is within the scope of these Guidelines, and not specifically exempt from them, complete a proposal form (obtainable from the IBC) giving full details of the proposed work and attaching supporting papers, if necessary;

XII.D.1.3 Forward the top two copies of the proposal form to the IBC of the institution, or institutions, where the work will be done.(*)

Work assessed by the principal investigator as Category VI.C.2 or VI.C.3 may commence after a proposal has been submitted to the IBC.

(*) **Where no IBC exists in the institution where the work will be done, work must not commence until an IBC has been formed and registered with the NBTAC and the laboratory certified. It is possible for the IBC of a neighboring institution to take responsibility for supervision of the work. (see Section II.B)**

Work must be conducted at a minimum of BL1 level of physical containment in a certified BL1 facility.

A proposal requesting special exemption from the Guidelines IV.C.1 should attach a submission detailing the case for less stringent conditions. While waiting for the IBC's decision work may commence at BL2 level of physical containment in a certified BL2 facility.

The work evaluated by the principal investigator as Categories VI.C.1 or VI.C.2, may not commence without the specific approval of the IBC or an exemption.

XII.E INSTITUTIONAL BIOSAFETY COMMITTEE

When the IBC receives a completed proposal form it must:

XII.E.1 Examine the details provided to ensure that all required information has been provided;

XII.E.2 Using the IBC Assessment form (see Appendix F) assess the proposal in terms of:

XII.E.2.1 Category of recombinant DNA work (as per Section VI.C);

XII.E.2.2 Required physical containment level;

XII.E.2.3 Availability of the required facilities to the principal investigator;

XII.E.2.4 Experience and training of the workers in the project team;

XII.E.2.5 Any special safety requirements; and

XII.E.3 either, endorse the proposal (perhaps after seeking further information) and within three weeks of endorsement send a copy with the completed assessment to the NBTAC for information;

XII.E.4 or, send a copy of the proposal with the assessment direct to the NBTAC with a request for advice on specified points.(*);

XII.E.5 Inform the principal investigator of its decision/action.

M **Proposals sent requesting advice to the NBTAC will be forwarded immediately to the Scientific Sub-Committee asking for a recommendation and the Sub-Committee will forward them to the IBC as soon as possible.**

XII.F ROLES AND RESPONSIBILITIES OF THE PRINCIPAL INVESTIGATOR

The Principal Investigator must be thoroughly familiar with the requirements of these Guidelines and must ensure that, where applicable, they are followed in respect to any project involving the use of the recombinant DNA technique for which he/she is responsible. In particular he/she must ensure that:

XII.F.1 The top two copies of a completed Recombinant DNA Committee proposal form are submitted to the IBC responsible for the laboratories where the work is to be carried out before starting any work on the project.

XII.F.2 Anew proposal form is forwarded to the IBC before any substantial change is made to the components of the donor DNA or host/vector system.

XII.F.3 The work is carried out under the approved conditions of physical containment.

XII.F.4 Personnel are aware of the nature of the work and have received appropriate training.

XII.F.5 All changes to the project team and equipment are notified to the IBC.

XII.F.6 All accidents and unexplained illnesses or absences are immediately reported to the IBC.

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rDNA BIOTECHNOLOGY
GLOSSARY

A

Acclimatization	**Adaptation of an organism to a new environment**
Active immunity	**A type of acquired immunity whereby resistance to a disease is built up by either having the disease or receiving a vaccine to it.**
Adjuvant	**Insoluble material that increases the formation and persistence of antibodies when injected with an antigen.**
Aerobic	**Needing oxygen for growth.**
Aerosol	**A suspension of fine liquid particles in a gas.**
Affinity chromatography	**A technique used in bioprocess engineering for separation and purification of almost any biomolecule on the basis of its biological function or chemical structure. The molecule to be purified is specifically and reversibly adsorbed by a complementary binding substance (ligand) and immobilized on a matrix. The substance of interest is first bound to the Immobilized ligand and then dissociated to recover by changing experimental conditions.**
Agglutinin	**An antibody that, when stimulated by the presence of the appropriate antigen, causes the clumping (agglutination) of bacteria or other cells.**
Allele	**Any of several alternative forms of a gene.**
Allelopathic agents	**Substances produced by plants that inhibit the growth of other plants or Insects, thus acting as natural herbicides or pesticides.**
Allogenic	**Of the same species, but with a different genotype.**
Amino acids	**Building blocks of proteins. There are twenty common amino acids: alanine, arginine, aspargine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.**
Amplification	**The process of Increasing the number of copies of a particular gene or chromosomal sequence.**
Anaerobic	**Growing in the absence of oxygen.**
Antibiotic	**Chemical substance formed as a metabolic by-product in bacteria or fungi and used to treat bacterial Infections. Antibiotics can be produced naturally, using microorganisms, or synthetically.**
Antibody	**Protein produced by humans and higher animals in response to the presence of a specific antigen.**

Anticodon — Triplet of nucleotide bases (codon in transfer RNA that pairs with - is complementary to a triplet in messenger RNA.) For example, if the codon is UCG, the anticodon is AGC. See also Base; Base pair; Complementarity.

Antigen — A substance that, when introduced Into the body, induces an immune response by a specific antibody.

Antigenic determinant — See Hapten.

Antihemophilic factors — A family of whole-blood proteins that initiate blood clotting. Some of these proteins, such as Factor VIII, can be used to treat hemophilia. See also Factor VIII; kidney plasminogen activator.

Antiserum — Blood serum containing specific antibodies against an antigen. Antisera are used to confer passive immunity to many diseases.

Assay — Technique for measuring a biological response.

Attenuated — Weakened; with reference to vaccines, made from pathogenic organisms that have been treated so as to render them avirulent.

Autoimmune disease — A disease in which the body produces antibodies against its own tissues.

Autoimmunity — A condition in which the body mounts an immune response against one of its own organs or tissues.

Autosome — Any chromosome other than a sex chromosome.

Auxotrophy — Requirement by a mutant micro-organism for growth factors not needed by the corresponding wild type micro-organism.

Avirulent — Unable to cause disease.

B

Bacillus subtilis — A bacterium commonly used as a host in recombinant DNA experiments. Important because of its ability to secrete proteins.

Bacteriophage — Virus that lives in and kills bacteria. Also called phage.

Bacterium — Any of a large group of microscopic organisms with a very simple cell structure. Some manufacture their own food, some live as parasites on other organisms, and some live on decaying matter.

Base — On the DNA molecule, one of the four chemical units that, accordingly to their order and pairing, represent the different amino acids. The four

	bases are: adenine (A), cytosine (C), guanine (G), and thymine (T), in RNA, uracil (U) substitutes for thymine.
Base pair	Two nucleotide bases on different strands of the nucleic acid molecule that bond together. The bases can pair in only one way: adenine with thymine (DNA) or uracil (RNA), and guanine with cytosine.
Batch processing	Growth in a closed system with a specific amount of nutrient medium. In bioprocessing, defined amounts of nutrient material and living matter are placed in a bioreactor and removed when the process is completed. See also: Continuous processing.
Bioassay	Determination of the effectiveness of a compound by measuring its effect on animals, tissues, or organisms in comparison with a standard preparation.
Biocatalyst	In bioprocessing, an enzyme that activates or speeds up a biochemical reaction.
Biochemical	The product of a chemical reaction in a living organism.
Biochip	Electronic device that uses organic molecules to form a semiconductor.
Biocide	See Bactericide.
Bioconversion	Chemical restructuring of raw materials by using a biocatalyst.
Biodegradable	Capable of being broken down by the action of microorganisms.
Biological oxygen demand (BOD)	The amount of oxygen used for growth by organisms in water that contains organic matter.
Biologic response modulator	A substance that alters the growth or functioning of a cell. Includes hormones and compounds that affect the nervous and immune systems.
Biological containment	Characteristics of an organism which limit its survival and/or multiplication in an environment.
Biomass	The totality of biological matter in a given area. As commonly used in biotechnology, refers to the use of cellulose, a renewable resource, for the production of chemicals that can be used to generate energy or al aternative feedstocks for the chemical industry to reduce dependence on nonrenewable fossil fuels.
Biopolymer	Naturally occurring macromolecule that include proteins, nucleic acids, and polysaccharides.

Bioprocess	**A process in which living cells, or components thereof, are used to produce a desired end product.**
Bioreactor	**Vessel used for bioprocessing.**
Biosynthesis	**Production of a chemical by a living organism.**
Biosynthetic process	**The process by which chemical compounds are produced by a living organism either by synthesis or degradation.**
Biota	**The flora and fauna of a region.**
Biotechnology	**Development of products by a biological process. Production may be carried out by using intact organisms, such as yeasts and bacteria, or by using natural substances (e.g., enzymes) from organisms.**
B lymphocytes (B-cells)	**A class of lymphocytes, released from the bone marrow, which produce antibody.**

C

Callus	**A cluster of undifferentiated plant cells that can, in some species, be induced to form the whole plant.**
Carcinogen	**Cancer-causing agent.**
Catalyst	**An agent (such as an enzyme or a metallic complex) that facilitates a reaction but is not itself changed during the reaction.**
Cell	**The smallest structural unit of living organisms that is able to grow and reproduce independently.**
Cell culture	**Growth of cells under laboratory conditions.**
Cell fusion	**See Fusion.**
Cell line	**Cells which grow and replace continuously outside the living organism.**
Cell-mediated immunity	**Acquired Immunity In which T lymphocytes play a predominant role. Development of the thymus in early life is critical to the proper development and functioning of cell-mediated immunity.**
Chemostat	**Growth chamber that keeps a bacterial culture at a specific volume and rate of growth by continually adding fresh nutrient medium while removing spent culture.**
Chimera	**The individual (animal or lower organism) produced by grafting an embryonic part of one species onto an embryo of either the same or a different species.**
Chloroplasts	**Cellular organelles where photosynthesis occurs.**

Chromosomes	**Threadlike components in the cell that contain DNA and proteins. Genes are carried on the chromosomes.**
Cistron	**A length of chromosomal DNA representing the smallest functional unit of heredity, essentially Identical to a gene.**
Clone	**A group of genes, cells, or organisms derived from a common ancestor. Because there is no combining of genetic material (as in sexual reproduction), the members of the clone are genetically identical to the parent.**
Codon	**A sequence of three nucleotide bases that specifies an amino acid or represents a signal to stop or start a function.**
Coenzyme	**An organic compound that is necessary for the functioning of an enzyme. Coenzymes are smaller than the enzymes themselves and sometimes separable from them.**
Cofactor	**A nonprotein substance required for certain enzymes to function. Cofactors can be coenzymes or metallic ions.**
Colonisation	**The establishment of a population within a new territory, e.g., the establishment of a novel colony of micro-organisms in the gastrointestinal tract.**
Colony-stimulating factors	**A group of lymphokines which induce the maturation and proliferation of white blood cells from the primitive cell types present in bone marrow.**
Complemetarity	**The relationship of the nucleotide bases on two different strands of DNA or RNA. When the bases are paired properly (adenine with thymine (DNA) or uracil (RNA; guanine with cytosine), the strands are complementary.**
Complementary DNA (cDNA)	**DNA synthesized from a messenger RNA rather than from a DNA template. This type of DNA is used for cloning or as a DNA probe for locating specific genes in DNA hybridization studies.**
Conjugation	**Sexual reproduction of bacterial cells in which there is a one-way exchange of genetic material between the cells in contact.**
Continuous processing	**A method of bioprocessing in which new materials are added and products removed continuously at a rate that maintains the volume at a specific level.** Of. **Batch processing.**
Cross-breeding	**To interbreed two varieties or breeds of the same species.**

Crossing over	**Exchange of genes between two paired chromosomes.**
Culture	**As a noun, cultivation of living organisms in prepared medium; as a verb, to grow in prepared medium.**
Culture medium	**Any nutrient system for the artificial cultivation of bacteria or other cells; usually a complex mixture of organic and Inorganic materials.**
Cyto-	**Referring to cell or cell plasm.**
Cytogenetics	**Study of the cell and its heredity-related components, especially chromosomes.**
Cytoplasm	**Cellular material that is within the cell membrane and surrounds the nucleus.**
Cytotoxic	**Able to cause cell death.**

D

Deamination	**Removal of amino (NH2) groups.**
Dehalogenation	**Removal of halogen (e.g. C12,12) groups.**
Denitration	**To remove nitric acid nitrates, the nitro group, or nitrogen oxides.**
DNA	**Deoxyribonucleic acid; polymer composed of deoxyribonucleotide units; genetic material of all organisms except RNA viruses.**
Deoxyribonucleic acid (DNA)	**The molecule that carries the genetic information for most living systems. The DNA molecule consists of four bases (adenine, cytosine, guanine, and thymine) and a sugar-phosphate backbone, arranged in two connected strands to form a double helix. See also Complementary DNA; Double helix; Recombinant DNA.**
Differentiation	**The process of biochemical and structural changes by which cells become specialized in form and function.**
Diploid	**A cell with two complete sets of chromosomes. See also: Haploid.**
DNA	**See Deoxyribonucleic acid.**
DNA probe	**A molecule (usually a nucleic acid) that has been labeled with a radioactive isotope, dye, or enzyme and is used to locate a particular nucleotide sequence or gene on a DNA molecule.**
DNA sequence	**The order of nucleotide bases in the DNA molecule.**

Double helix	A term often used to describe the configuration of the DNA molecule. The helix consists of two spiraling strands of nucleotides (a sugar, phosphate, and base), joined crosswise by specific pairing of the bases. See also Deoxyribonucleic; Base; Base pair.
Downstream processing	The stages of processing that take place after the fermentation or bioconversion stage. Includes separation, purification, and packaging of the product.

E

Ecosystem	The complex of a community and its environment functioning as an ecological unit in nature.
Electron transfer proteins	Proteins involved in the sequential transfer of electrons, (especially in cellular respiration) from an oxidisable substrate to molecular oxygen by a series of oxidation-reduction reactions.
Electrophoresis	A technique for separating different types of molecules based on their patterns of movement in an electrical field.
Endonuclease	An enzyme that breaks nucleic acids at specific interior bonding sites, thus producing nucleic acid fragments of various lengths. See also: Exonuclease.
Enzyme	A protein catalyst that facilitates specific chemical or metabolic reactions necessary for cell growth and reproduction.
Epidemiological	Concerning the incidence, distribution and control of organisms, particularly a pathogen.
Escherichia coli	A bacterium that inhabits the intestinal tract of most vertebrates. Much of the work using recombinant DNA techniques has been carried out with this organism because it has been genetically well characterized.
Eukaryote	A cell or organism containing a true nucleus, with a well-defined membrane surrounding the nucleus. All organisms except bacteria, viruses, and blue-green algae are eukaryotic. See also: Prokaryote.
Exon	In eukaryotic cells, the part of the gene that is transcribed into messenger RNA and encodes a protein. See also intron; Splicing.
Exonuclease	An enzyme that breaks down nucleic acids only at the ends of polynucleotide chains, thus releasing

one nucleotide at a time, in sequential order. See also: Endonuclease.

Expression — In genetics, manifestation of a characteristic that is specified by a gene. With hereditary diseases, for example, a person can carry the gene for the disease but not actually have the disease. In this case, the gene is present but not expressed. In industrial biotechnology, the terms is often used to mean the production of a protein by a gene that has been inserted into a new host organism.

F

Factor VIII — A large, complex protein that aids in blood clotting and is used to treat hemophilia. See also Antihemophilic factors.

Feedstock — The raw material used for chemical or biological processes.

Fermentation — An anaerobic process of growing micro-organisms for the production of various chemical or pharmaceutical compounds. Microbes are normally incubated under specific conditions in the presence of nutrients in large tanks called fermentors.

Floculation — The agglomeration of suspended material to form particles that will settle by gravity, as in the 'tertiary' treatment of waste materials.

Frameshift — Insertion or deletion of one or more nucleotide bases such that incorrect triplets of bases are read as codons.

Fusion — Joining of the membrane of two cells, thus creating a daughter cell that contains the nuclear material from parent cells. Used in making hybridomas.

G

Gene — A segment of chromosome. Some genes direct the synthesis of proteins while others have regulatory functions. See also Operator g.; Regulatory g.; Structural g.; Suppressor g.

Gene machine — A computerized device for synthesizing genes by combining nucleotide (bases) in the proper order.

Gene mapping — Determination of the relative locations of genes on a chromosome.

Gene probe	A specific DNA or RNA sequence used to detect complementary sequences among nucleic acid molecules.
Gene sequencing	Determination of the sequence of nucleotide bases in a strand of DNA.
Genetic code	The mechanism by which genetic information is stored in living organisms. The code uses sets of three nucleotide bases (codons) to make the amino acids that, in turn, constitute proteins.
Genetic engineering	A technology used to alter the genetic material of living cells in order to make them capable of producing new substances or performing new functions.
Genetic material	DNA, genes, chromosomes which constitute an organism's hereditary material; RNA in certain viruses.
Genome	The total hereditary material of a cell, comprising the entire chromosomal set found in each nucleus of a given species.
Genotype	Genetic make-up of an individual or group. See also: Phenotype.
Germ cell	Reproductive cell (sperm or egg). Also called gamete or sex cell.
Germicide	See Bactericide.
Germplasm	The total genetic variability, represented by germ cells or seeds, available to a particular population of organisms.

H

Haploid	A cell with half the usual number of chromosomes, or only one chromosome set. Sex cells are haploid. See also: Diploid.
Hapten	The portion of an antigen that determines its immunological specificity. When coupled to a large protein, a hapten stimulates the formation of antibodies to the two-molecule complex. Also called antigenic determinant.
Hemagglutination	Clumping (agglutination) of red blood cells.
Heredity	Transfer of genetic information from parent cells to progeny.
	grafting can be done without tissue rejection.

Histocompatibility antigen	**An antigen that causes the rejection of grafted material from an animal different in genotype from the host animal.**
Homologous	**Corresponding or alike in structure, position, or origin.**
Hormone	**A chemical that acts as a messenger or stimulatory signal, relaying instructions to stop or start certain physiological activities. Hormones are synthesized in one type of cell and then released to direct the function of other cell types.**
Host	**A cell or organism used for growth of a virus, plasmid, or other form of foreign DNA, or for the production of cloned substances.**
Host-vector system	**Combination of DNA-receiving cells (host) and DNA-transporting substance (vector) used for Introducing foreign DNA into a cell.**
Humoral immunity	**Immunity resulting from circulating antibodies in plasma protein.**
Hybridization	**Production of offspring, or hybrids, from genetically dissimilar parents. The process can be used to produce hybrid plants (by cross-breeding two different varieties) or hybridomas (hybrid cells formed by fusing two unlike cells, used in producing monoclonal antibodies). The terms is also used to refer to the binding of complementary strands of DNA or RNA**
Hybridoma	**The cell produced by fusing two cells of different origin. In monoclonal antibody technology, hybridomas are formed by fusing an immortal cell (one that divides continuously) and an antibody-producing cell. See also Monoclonal antibody; Myeloma.**
Immune serum	**Blood serum containing antibodies.**
Immune system	**The aggregation of cells, biological substances (such as antibodies), and cellular activities that work together to provide resistance to disease.**
Immunity	**Nonsusceptibility to a disease or to the toxic effects of antigenic material. See also Active i.; Cell-mediated i.; Humors[i.; Natural active i.; Natural passive I.; Passive i.**
Immunoassay	**Technique for identifying substances based on the use of antibodies.**

Immunofluorescence	Technique for identifying antigenic material that uses antibody labeled with fluorescent material. Specific binding of the antibody and antigen can be seen under a microscope by applying ultraviolet light rays and noting the visible light that is produced.
Immunogen	Any substance that can elicit an immune response.
Immunoglobulin	General name for proteins that function as antibodies. These proteins differ somewhat in structure, and are grouped into five categories on the basis of these differences: immunoglobulin G (IgG), IgM, IgA, and IgE.
Immunology	Study of all phenomena related to the body's response to antigenic challenge (i.e., immunity, sensitivity, and allergy).
Inducer	A molecule or substance that increase the rate of enzyme synthesis, usually by blocking the action of the corresponding repressor.
Insectary	Place for the keeping or rearing of living insects.
Interferon	A class of lymphokine proteins important in the immune response. There are three major types of interferon: alpha (leukocyte), beta (fibroblast), and gamma (immune). Interferons inhibit viral infections and may have anticancer properties.
Interleukin	A type of lymphokine whose role in the immune system is being extensively studied. Two types of interleukin have been identified. Interleukin I (IL-1), derived from macrophages, is produced during inflammation and amplifies the production of other lymphokines, notably interleukin 2(IL-2), IL-2 regulates the maturation and replication of T lymphocytes.
Intron	In eukaryotic cells, a sequence of DNA that is contained in the gene but does not encode for protein. The presence of introns "splits" the coding region of the gene into segments called exons. See also Exon; Splicing.
In vitro	Literally, "in glass." Performed in a test tube or other laboratory apparatus.
In vivo	In the living organism.
Isoenzyme (isozyme)	One of the several forms that a given enzyme can take. The forms may differ in certain physical properties, but function similarly as biocatalysts.
Isogenic	Of the same genotype.

K

Kidney plasminogen activator	**A precursor to the enzyme urokinase that has blood-clotting properties.**

L

Leaching	**The removal of a soluble compound such as an ore from a solid mixture by washing or percolating.**
Leukocyte	**A colorless cell in the blood, lymph, and tissues that is an important component of the body's immune system; also called white blood cell.**
Library	**A set of cloned DNA fragments.**
Ligase	**An enzyme used to join DNA or RNA segments together. They are called DNA ligase or RNA ligase, respectively.**
Linkage	**The tendency for certain genes to be inherited together due to their physical proximity on the chromosome.**
Linker	**A fragment of DNA with a restriction site that can be used to join DNA strands.**
Lipopolysaccharide	**A water-soluble lipid-polysaccharide complex.**
Lymphocyte	**A type of leukocyte found in lymphatic tissue in the blood, lymph nodes, and organs. Lymphocytes are continuously made in the bone marrow and mature into antibody-forming cells. See also B lymphocytes; T lymphocytes.**
Lymphokine	**A class of soluble proteins produced by white blood cells that play a role, as yet not fully understood, in the immune response. See also Interferon; Interleukin.**
Lymphoma	**Form of cancer that affects the lymph tissue.**
Lysis	**Breaking apart of cells.**
Lysozyme	**An enzyme present in, for example, tears, saliva, egg whites, and some plant tissues that destroys the cells of certain bacteria.**

M

Macrophage	**A type of white blood cell produced in blood vessels and loose connective tissues that can ingest dead tissue and cells and is involved in producing interleukin 1. When exposed to the lymphokine "macrophage-activating factor,"**

	macrophages also kill tumor cells. See also Phagocyte.
Macrophage-activating factor	An agent that stimulates macrophages to attack and ingest cancer cells.
Medium	A substance containing nutrients needed for cell growth.
Meiosis	Process of cell reproduction whereby the daughter cells have half the chromosome number of the parent cells. Sex cells are formed by meiosis. See also: Mitosis.
Messenger RNA (mRNA)	Nucleic acid that carries Instructions to a ribosome for the synthesis of a particular protein.
Metabolism	All biochemical activities carried out by an organism to maintain life.
Metazoan cell	Cell from a multicellular (metazoan) organism rather than unicellular (protozoan) organism.
Microbiology	Study of living organisms that can be seen only under a microscope.
Microcosm	A community which is a representation of a larger system.
Microinjection	The technique of introducing very small amounts of material (DNA or RNA) molecules, enzymes, cytctoic agents) into an intact cell through a microscopic needle penetrating the cell membrane.
Microorganism	Any organism that can be seen only with the aid of a microscope. Also called microbe.
Mitochondria	Structures in higher cells that serve as the "powerhouse" for the cell, producing chemical energy.
Mitosis	Process of cell reproduction whereby the daughter cells are identical in chromosome number to the parent cells. See also: Meiosis.
Modulators of the immune system	Non-antibody proteins released by primed lymphocytes on contact with antigen which act as intercellular mediators of the immune response.
Molecular genetics	Study of how genes function to control cellular activities.
Monoclonal antibody	Highly specific, purified antibody that is derived from only one done of cells and recognizes only one antigen. See also Hybridoma; Myeloma.
mRNA	Messenger RNA
Multigenic	Of hereditary characteristics, one that is specified by several genes.

Mutagen	A substance that induces mutations.
Mutagenesis	The induction of mutation in the genetic material of an organism; researchers may use physical or chemical means to cause mutations that improve the production capabilities of organisms.
Mutant	A cell that manifests new characteristics due to a change in its DNA.
Mutation	A change in the genetic material of a cell.
Muton	The smallest element of a chromosome whose alteration can result in a mutation or a mutant organism.
Myeloma	A type of tumor cell that is used in monoclonal antibody technology to form hybrrdomas.

N

Natural active immunity	Immunity that is established after the occurrence of a disease.
Natural killer (NK) cell	A type of leukocyte that attacks cancerous or virus-infected cells without previous exposure to the antigen. NK cell activity is stimulated by interferon.
Natural passive immunity	Immunity conferred by the mother on the fetus or newborn.
Nitrogen fixation	A biological process (usually associated with plants) whereby certain bacteria convert nitrogen in the air to ammonia, thus forming a nutrient esential for growth.
Non-viable	Not capable of living, growing, or developing and functioning successfully.
Nuclease	An enzyme that, by cleaving chemical bonds, breaks down nucleic acids into their constituent nucleotides. See also Exonuclease.
Nucleic acids	Large molecules, generally found in the cell's nucleus and/or cytoplasm, that are made up of nucleotide bases. The two kinds of nucleic acid are DNA and RNA
Nucleotide base	See Base.
Nucleotides	The building blocks of nucleic acids. Each nucleotide is composed of sugar, phosphate, and one of four nitrogen bases. The sequence of the bases within the nucleic acid determines what proteins will be made.
Nucleus	The structure within eukaryotic cells that contains chromosomal DNA.

O

Oligonucleotide	A polymer consisting of a small number (about two to ten) of nucleotides.
Oncogene	Gene thought to be capable of producing cancer.
Oncogenic	Cancer causing.
Oncology	Study of tumors.
Operator gene	A region of the chromosomes, adjacent to the operon, where a repressor protein binds to prevent transcription of the operon.
Operon	Sequence of genes responsible for synthesizing the enzymes needed for biosynthesis of a molecule. An operon is controled by an operator gene and a repressor gene.
Opsonin	An antibody that renders bacteria and other antigenic material susceptible to destruction by phagocytes.
Organic compound	A compound containing carbon.
Organism	Any biological entity, cellular or non-cellular, with capacity for self-perpetuation and response to evolutionary forces; includes plants, animals, fungi, protists, prokaryotes, and viruses.

P

Passive immunity	Immunity acquired from receiving preformed antibodies.
Pathogen	Disease-causing organism.
Pathogenic	Capable of causing disease.
Peptide	Two or more amino acids joined by a linkage called a peptide bond.
Phage	See Bacteriophage.
Phagocyte	A type of white blood cell that can ingest invading microorganisms and other foreign material. See also Macrophage.
Phagocytosis	The engulfing and (usually) destruction of particulate matter by cells (such as the leukocyte) that characteriscally engulf foreign material and consume debris.
Phenotype	Observable characteristics, resulting from interaction between an organism's genetic make-up and the environment. See also: Genotype.

Photosynthesis	Conversion by plants of light energy into chemical energy, which is then used to support the plants' biological processes.
Physical containment	Procedures of structures designed to reduce or prevent the release of viable organisms; degree of containment varies.
Pituitary	A small oval endocrine organ that is attached to the infundibulum of the brain and produces various internal secretions directly or Indirectly impinging on most basic body functions.
Plasma	The fluid (noncellular) fraction of blood.
Plasmid	A small circular form of DNA that carries certain genes and is capable of replicating independently in a host cell.
Polyclonal	Derived from different types of cells.
Polymer	A long molecule of repeated subunits.
Polymerase	General term for enzymes that carry out the synthesis of nucleic acids.
Polynucleotide	A polymeric chain of compounds consisting of a ribose or deoxyribose sugar joined to a purine or pyrimidine base and a phosphate group and that are the basic structural units of DNA and RNA.
Polypeptide	Long chain of amino acids joined by peptide bonds.
Population	A body of individuals having a characteristic in common.
Probe	See DNA probe.
Prokaryote	An organism (e.g., bacterium, virus, blue-green algae) whose DNA is not enclosed within a nuclear membrane. See also: Eukaryote.
Promoter	A DNA sequence that is located in front of a gene and controls gene expression. Promoters are required for binding of RNA polymerase to initiate transcription.
Prophage	Phage nucleic acid that is incorporated into the host's chromosome but does not cause cell lysis.
Protein	A molecule composed of amino acids. There are many types of proteins, all carrying out a number of different functions essential for cell growth.
Protoplast	The cellular material that remains after the cell wall has been removed.
Pure culture	In vitro growth of only one type of microorganism.

R

Radioimmunoassay	**A technique for quantifying a substance by measuring the reactivity of radioactively labeled forms of the substance with antibodies.**
Reagent	**Substance used in a chemical reaction.**
Recombinant DNA (rDNA)	**The DNA formed by combining segments of DNA from different types of organisms.**
Regeneration	**Laboratory technique for forming a new plant from a clump of plant cells.**
Regulatory gene	**A gene that acts to control the protein-synthesizing activity of other genes.**
Replication	**Reproduction or duplication, as on an exact copy of a strand of DNA.**
Replicon	**A segment of DNA (e.g., chromosome or plasmid) than can replicate independently.**
Repressor	**A protein that binds to an operator adjacent to a structural gene, inhibiting transcription of that gene.**
Restriction enzyme	**An enzyme that breaks DNA in highly specific locations, creating gaps into which new genes can be inserted.**
Reticuloendothelial system	**The system of macrophages, which serves as an important defense system against disease.**
Retrovirus	**An animal virus with a glycoprotein envelope and an RNA genome that replicates through a DNA intermediate.**
Rheology	**Study of the flow of matter such as fermentation liquids.**
Ribonucleic acid (RNA)	**A molecule similar to DNA that functions primarily to decode the instructions for protein synthesis that are carried by genes. See also Messenger RNA; Transfer RNA**
Ribosome	**A cellular component, containing protein and RNA, that is involved in protein synthesis.**
Ring cleavage	**The splitting of a compound in which the molecules are arranged cyclically (a closed chain) commonly consisting of five or six atoms although smaller and larger rings are known.**
RNA	**Ribonucleic acid.**

S

Scale-up	**Transition from small-scale production to production of large industrial quantities.**
Sclerotia	**Fungal survival structures capable of remaining dormant for long periods.**
Screening	**To select organisms on the basis of a specific characteristic.**
Secondary	**Metabolite that is not required by the producing organism for its life-support system.**
Selection	**A laboratory process by which cells or organisms are chosen for specific characteristics.**
Selective medium	**Nutrient material constituted such that it will support the growth of specific organisms while inhibiting the growth of others.**
Serology	**Study of blood serum and reactions between the antibodies and antigens therein.**
Single-cell protein	**Cells or protein extracts from microorganisms, grown In large quantities for use as protein supplements.**
Somatic cells	**Cells other than sex or germ cells.**
Splicing	**The removal of introns and joining of exons to form a continuous coding sequence in RNA.**
Spore	**A dormant cellular form, derived from a bacterial or a fungal cell, that is devoid of metabolic activity and that can give rise to a vegetative cell upon germination; it is dehydrated and can survive for prolonged periods of time under drastic environmental conditions.**
Storage Protein Genes	**Genes coding for the major proteins found in plant seeds.**
Structural gene	**A gene that codes for a protein, such as an enzyme.**
Substrate	**Material acted on by an enzyme.**
Suppressor gene	**A gene that can reverse the effect of a mutation in other genes.**
Symbiont	**An organism living In symbiosis, usually the smaller member of a symbiotic pair of dissimilar size.**
Symbiotic	**Capable of living in an Intimate association with a dissimilar organism in a mutually beneficial relationship.**

T

Template	**A molecule that serves as the pattern for synthesizing another molecule.**
Thymus	**A lymphoid organ in the lower neck, the proper functioning of which in early life is necessary for development of the immune system.**
Tissue culture	**In vitro growth in nutrient medium of cells isolated from tissue.**
Tissue-type plasminogen activator	**A protein produced in small amounts in the body that aids in dissolving blood clots.**
T lymphocytes (T-cells)	**White blood cells that are produced in the bone marrow but mature in the thymus. They are important in the body's defense against certain bacteria and fungi, help B lymphocytes make antibodies, and help in the recognition and rejection of foreign tissues. T lymphocytes may also be important in the body's defense against cancers.**
Toxin	**A poisonous substance produced by certain microorganisms.**
Toxoid	**Detoxified toxin, but with antigenic properties intact.**
Transcription	**Synthesis of messenger (or any other) RNA on a DNA template.**
Transduction	**Transfer of genetic material from one cell to another by means of a virus or phage vector.**
Transfection	**Infection of a cell with nucleic acid from a virus, resulting in replication of the complete virus.**
Transfer RNA (tRNA)	**RNA molecules that carry amino acids to sites on nbosomes where proteins are synthesized.**
Transformation	**Change in the genetic structure of an organism by the incorporation of foreign DNA**
Transgenic animals	**Animals into which DNA from another species are introduced by microinjection or retroviral infection.**
Translation	**Process by which the information on a messenger RNA molecule is used to direct the synthesis of a protein.**
Translocation	**The exchange of parts between non-homologous chromosomes.**
Transposon	**A segment of DNA that can move around and be inserted at several sites in bacterial DNA or in a phage, thus altering the host's DNA**
tRNA	**Transfer RNA.**

Tumor necrosis factors	**Rare proteins of the immune system that appear to destroy some types of tumor cells without affecting healthy cells.**

V

Vaccine	**A preparation that contains an antigen consisting of whole disease-causing organisms (killed or weakened), or parts of such organisms, and is used to confer immunity against the disease that the organisms cause. Vaccine preparations can be natural, synthetic, or derived by recombinant DNA technology.**
Vector	**The agent (e.g., plasmid or virus) used to carry new DNA into a cell.**
Virion	**An elementary viral particle consisting of genetic material and a protein covering.**
Vroid	**Small pathogenic RNA molecule, apparently unable to code for protein, which depends on the host machinery for its replication.**
Vrion	**An elementary viral particle consisting of genetic material and a protein covering.**
Virology	**Study of viruses.**
Virulence	**Ability to infect or cause disease.**
Virus	**A submicroscopic organism that contains genetic information but cannot reproduce itself. To replicate, it must invade another cell and use parts of that cell's reproductive machinery.**

W

White blood cells	**Leukocytes.**
Wild type	**The form of an organism that occurs most frequently in nature.**

Y

Yeast	**A general term for single-celled fungi that reproduce by budding. Some yeasts can ferment carbohydrates (starches and sugars), and thus are important in brewing and baking.**

Z

Zygote	**A cell formed by the union of two mature reproductive cells.**

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APPENDIX F - PROPOSAL FORMS

Appendix F.1--Proposal Form for Assessment of Small Scale Work with Recombinant *DNA*

Work on a small scale (i.e. less than 10 litres of culture) with recombinant DNA molecules is subject to the provisions of Section VI.C of these Guidelines which specifies different categories of recombinant DNA work.

Work involving larger volumes of culture should be covered in the future by the Guidelines for Large Scale Work with Recombinant DNA and investigators should use the new proposal form for assessment of large scale work.

Appendix F.1.1--Submission of Proposals

Work assessed by the principal investigator as Category VI.C.3 or VI.C.4 does not require submission of a proposal.

A proposal must be submitted to the Institutional Biosafety Committee (IBC) responsible for the laboratories where the work is to be carried out before work commences for all work assessed by the principal investigator as Categories VI. C.1 or VI.C.2. Where the work is carried out in more than one organization both IBCs must be informed.

Investigators planning to work with whole plants must also submit a supplementary information form for recombinant DNA work involving whole plants. A copy of this form may be found in Appendix F.5 of these Guidelines.

Appendix F.1.2-Approval of Proposals and Commencement of Work

Work assessed by the principal investigators Category VI.C.2 may commence after a proposal has been submitted to the IBC. Work must be conducted at a minimum of BL1 level of physical containment.

A proposal requesting special exemption from the Guideline under Category VI.C.1 should attach a submission detailing the case for less stringent conditions. While waiting for the IBC's decision, work may commence at BL2 level of physical containment.

Work assessed by the principal investigator as Categories VI. C.1 and VI.C.2 may not commence without the specific approval of the IBC following a recommendation from the NBTAC Scientific Sub-Committee.

Appendix F.1.3--The Proposal Form

This form must be signed by the principal investigator before submission to the IBC.

The top two copies are to be forwarded to the IBC, which will send one copy to the RDNA-C Scientific Sub-Committee.

The IBC will check the information provided regarding the proposed biological system, the physical containment facilities to be used and the details of the members of the project team. It will then make its evaluation of the proposal with regard to the proposed level of physical containment and the adequacy of the experience of the members of the team for carrying out the proposed work.

Appendix F.1.4--Confidential Information

Applicants who wish to restrict access to the information provided on the form should mark the form and any attachments "Commercial in Confidence".

MODEL PROPOSAL FORM (APPENDIX F.1)

IBC Reference Number: . . . . . .

NBTAC reference number: . . . . . .

APPENDIX F.2 PROPOSAL FORM FOR ASSESSMENT OF SMALL SCALE WORK WITH RECOMBINANT DNA

Before completing this form please read the instructions on Appendix F.1. If insufficient space is provided please attach additional pages.

Appendix F.2.1

Name and full professional address of principal investigator submitting proposal.

Appendix F.2.2

Names of other principal investigators responsible for the project. Please give their professional address if different from that in (1) above.

Appendix F.2.3

Title of Project

Appendix F.2.4

Describe the aim of the work.

Appendix F.2.5

Details of Biological System

NOTE: Any substantial change in the parameters of the system will require submission of another proposal. Give detailed information of the biological system as requested below. Attach any relevant papers or further information which will assist in evaluating any potential hazard which may be associated with this work, such as information regarding any special biological containment features of the host species or toxins produced by the donor species.

Appendix F.2.5.a

Describe the biological source of the donor DNA to be used - include the genus, species and strain or organ/tissue as applicable.

Appendix F.2.5.b

Describe the host organism or tissue to be used - include the genus, species and strain where applicable. If not a commonly used laboratory strain include the name of the strain from which it is derived.

Appendix F.2.5.c

Describe the vectors to be used to transfer donor DNA to the host. If symbols or numbers are used to name the vectors, include information regarding the origin of the vector. Include a full description of any retroviral vectors.

Appendix F.2.5.d

What is the classified level of biological containment offered by the host/vector system(s) (see Appendix E)

HV1 HV2 Approved Not classiffied

Appendix F.2.6

Into which category of work in Section VI.C of the Guidelines does this work fall?

Appendix F.2.7

Where will this work be conducted? Give building and room number

Appendix F.2.8

What is the certified containment level of this facility? (see Appendix F.II and D.IV)

BL1 BL2 BL3 BL4 Other

Appendix F.2.9

Do you have approval to use this facility? Attach written confirmation if not located at your professional place of work.

Appendix F.2.10

Proposed date of commencement of work. Likely duration of work.

Appendix F.2.11

Details of personnel involved with this project. For each person working on this project attach full details of name, qualifications, microbiological or other relevant experience and role in the project team on a separate sheet of paper.

Appendix F.2.12

Signature of Principal Investigator submitting this proposal

Date: .....

APPENDIX F.3 INSTITUTIONAL BIOSAFETY COMMITTEE ASSESSMENT OF A PROPOSAL TO DO SMALL SCALE RECOMBINANT DNA WORK

This form is to be completed by the Institutional Biosafety Committee (IBC) following receipt of a Proposal Form for Assessment of Small Scale Work with Recombinant DNA.

In completing this form reference should be made to the relevant sections of the Guidelines for Small Scale Work with Recombinant DNA.

Appendix F.3.1

Procedure Following Completion of Assessment Form

Appendix F.3.1.1

Proposals assessed as Class VI.C.1:

Attach the top copy of the completed assessment form to the top copy of the proposal form (Appendix F.1 and F.2) and send to the NBTAC Secretariat as soon as possible.

Advise the applicant that work may not commence until the IBC has approved the project following receipt of advice from the NBTAC Scientific Sub-Committee.

Appendix F.3.1.2

Proposals assessed as Class VI.C.2:

Attach the top copy of the completed assessment form to the top copy of the proposal form (Appendix F.1 and F.2) and send to the NBTAC Secretariat within three weeks of endorsement of the project.

Advise the applicant ofthe IBC's endorsement of the project.

Appendix F.3.1.3

Proposals assessed as Class VI.C.3:

Information on these projects is not required by the NBTAC.

Proposals seeking special exemption under Classes VI.C.1 and VI.C.2.

Attach the top copy of the completed assessment form and the exemption submission to the top copy of the proposal form (Appendix F.1 and F.2) and send to the NBTAC Secretariat as soon as possible.

NOTE: The IBC Chairman must sign the appropriate section of the assessment form before it is sent to the NBTAC Secretariat. Forms which have not been signed will be returned to the IBC for signature.

MODEL PROPOSAL FORM (APPENDIX F.3)

NBTAC Reference .....

APPENDIX F.4 INSTITUTIONAL BIOSAFETY COMMITTEE ASSESSMENT OF A PROPOSAL TO DO SMALL SCALE RECOMBINANT DNA WORK

Section A - IBC Assessment

Appendix F.4.1

IBC Reference Number for this proposal

Appendix F.4.2

Names of principal investigators

Appendix F.4.3

Project title

Appendix FAA

The following information has been checked (please tick)

Appendix F.4.4.a

the biological system **Yes**

Appendix F.4.4.b

the physical containment facilities available **Yes**

Appendix F.4.4.c

the details regarding the personnel

involved in the project **Yes**

If insufficient information has been provided return the proposal form to the investigator with a request to provide the required information.

Appendix F.4.5

The training and experience of the project team for carrying out this type of work are considered adequate. Yes

Appendix F.4.6

IBC assessment of:

Appendix F.4.6.a

the category of the work (please give the number within the category as per Section VI.C of the Guidelines)

Category VI. C.1 . . . . . Category VI.C.2 . . . . . .

(NOTE: Categories VI.C.3 and VI.C.4 are exempted)

Appendix F.4.6.b

the physical containment required (please tick)

BL1 BL2 BL3 BL4 Other (please specify)

Section B - *IBC Request for NBTAC Scientific Sub-Committee Advice*

Complete this section only if work is assessed as Categories VI.C.1 and VI.C.2.

Appendix F.4.7

The IBC has evaluated the attached proposal as above and is forwarding it to the NBTAC Scientific Sub-Committee for advice. Specific advice is required regarding:

Signed: ..... Date:.....

(IBC Chairman)

Institution:.....

Section C - IBC Decision

This Section may be completed immediately for all Categories VI.C.2 and VI.C.3 work or completed following receipt of NBTAC recommendation for Category VI. C.1 work.

This proposal has been evaluated as above and endorsed/approved by the IBC. The following conditions in addition to those specified in the Guidelines must be adhered to during the conduct of the work:

Signed: ..... Date: .....

(IBC Chairman)

Institution:.....

MODEL PROPOSAL FORM (APPENDIX F.5)

APPENDIX F.5 SUPPLEMENTARY INFORMATION FORM FOR RECOMBINANT DNA WORK INVOLVING WHOLE PLANTS

Two copies of this form must be attached to and submitted to the IBC with the top two copies of a completed small scale proposal form (Appendix F.1 and F.2).

Appendix F.5.1

Name of principal investigator/project supervisor submitting proposal:

Appendix F.5.2

Name of organization:

Appendix F.5.3

Title of project (as on small scale proposal form):

Appendix F.5.4

NBTAC reference number of this and/or related project (if known):

Appendix F.5.5

Describe the experimental system to be used (plant species,vector, etc.)

Appendix F.5.6

Are the experimental plants noxious weeds or closely related to species which are noxious weeds?

If "yes" please elaborate: . . . . . .

Appendix F.5.7

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

If "yes" please elaborate: . . . . . .

Appendix F.5.7.a

Give further information about the harmful agent:

Appendix F.5.7.b

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

Appendix F.5.8

Are recombinant plants to be grown?

If "yes" please elaborate: .....

Appendix F.5.8.a

What developmental stage will they reach?

Appendix F.5.8.b

Describe the techniques to be employed to contain any pollen, seed, spores, etc.

Appendix F.5.9

To be grown:

Appendix F.5-9.a

Is soil or soil substitute to be used (specify)?

Appendix F.5.9.b

How will you sterilize it?

Appendix F.5.10

Describe the facility to be used for cultivation of the plants. Include information such as location, proximity to containment laboratory, etc.

Appendix F.5.11

Give any additional information which may be relevant to the assessment of this work:

Appendix F.5.12

Signature of Principal
Investigator/Project Supervisor: . . . . . .

Date: .....

IBC EVALUATION OF THIS PROPOSAL

Please complete this section in addition to the IBC assessment form for small scale proposals.

Appendix F.5.13

IBC evaluation of the work: . . . . . .

Appendix F.5.14

Signature of Chairman: . . . . . .

Date: .....