DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
Bethesda, Maryland 20205

December 18, 1981

M E M O R A N D U M

TO: Interested Parties

FROM: Director, Office of Recombinant DNA Activities

SUBJECT: Proposed Revisions of NIH Guidelines for Research Involving Recombinant DNA Molecules

Comments are invited on two proposals for a major revision of the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules.

The Recombinant DNA Advisory Committee (RAC) has developed a proposed revision and recommended that it be published for comment. This proposal appears in the Federal Register dated December 4, 1981.

The major features of this proposal are:

1. The Guidelines would cease to be mandatory and would become a voluntary code of standard practice. The following requirements would be eliminated: that institutions have an Institutional Biosafety Committee (IBC), that investigators obtain prior approval from the IBC before beginning certain experiments, and that investigators obtain prior approval from NIH before beginning certain experiments. The section of the Guidelines specifying that noncompliance could lead to loss of NIH funds would also be eliminated.

2. Section III of the Guidelines giving containment levels would be greatly simplified, and most experiments currently requiring P2 or P3 containment would be recommended at P1.

3. The prohibitions section (I-D) of the Guidelines would be eliminated, although two of the current prohibitions (I-D-2 and I-D-5) would be retained as admonishments.

An alternative proposal appears as item 7 in the Federal Register notice of December 7, 1981.

The major features of this proposal are:

1. The Guidelines would continue to be mandatory for institutions receiving NIH funding. Certain experiments would require prior review by NIH, certain experiments would require prior review by an IBC, and certain experiments would require notice to an IBC simultaneously with initiation of the experiment.

2. Section III of the Guidelines would be reorganized and simplified. All experiments would fall into one of four classes. Physical containment requirements for some classes of experiments would be lowered.

3. Three of five current prohibitions (I-D-2, I-D-4, and I-D-5 in the current Guidelines) would be listed in a new section that would continue to require RAC review and NIH approval before initiation. Experiments currently falling under prohibition I-D-1 and I-D-6 could proceed after IBC approval.

Copies of the above mentioned Federal Register notices as well as a summary comparing the current Guidelines with the two proposals are enclosed. Comments on these proposals should be directed to the attention of the Director, Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4A52, Bethesda, Maryland 20205, U.S.A.

These proposals and comments on them will be considered by the RAC at its next meeting on February 8-9, 1982.

William J. Gartland, Jr., Ph.D.

*Indicates the three major changes in the RAC Proposal

SUMMARY OF PROPOSED CHANGES IN NIH GUIDELINES
FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES

Prepared by B. Talbot
12/8/81

Current Guideline Section	Topic	Current Guidelines	RAC Proposal (Fed. Reg. 12/4/81)	Gottesman Proposal (Fed. Reg. 12/7/81)
IV	RAC	Composition and procedures detailed for Recombinant DNA Advisory Committee (RAC).	Unchanged from current Guidelines.	Unchanged from current Guidelines.
IV	Roles and Responsibilities (Other than RAC Composition and Procedures)	Guidelines are mandatory for those receiving NIH support. Institutions must have an Institutional Biosafety Committee (IBC) with specified membership. Principal Investigators (PIs) must obtain prior approval from IBC before beginning certain experiments. PIs must obtain prior approval from NIH before beginning certain experiments. Roles and Responsibilities are specified for the Institution, the IBC, the Biological Safety Officer (BSO), the PI, and the NIH. Section IV-G of the Guidelines specifies that noncompliance can lead to loss of NIH funds.	*Guidelines cease to be mandatory and become a voluntary code of good practice. Requirements that institutions have an IBC, that investigators must obtain prior approval from the IBC before beginning certain experiments, that investigators must obtain prior approval from the NIH before beginning certain experiments, and the Section of the Guidelines specifying that noncompliance with the Guidelines could lead to loss of NIH funds, would all be eliminated. Current specified and responsibilities for the Institution, the IBC, BSO and PI would be eliminated. A new statement would be added: "Each institution conducting or sponsoring recombinant DNA research should take responsibility for monitoring its own activities in this area. Any unusual events that might be associated with the use of recombinant DNA molecules should be reported to the Director, NIH."	Unchanged from current Guidelines in all important respects. There would be minor changes in this Section of the Guidelines as necessary to reflect changes in other Sections.
I-D	Prohibitions	Five classes of experiments prohibited. Prohibitions override other Sections of the Guidelines.	*Prohibitions eliminated. Two of the current prohibitions (drug resistance traits and toxin genes) become admonishments in Part III of the Guidelines.	The term prohibition is no longer used. However, three of the current prohibitions (drug resistance traits, toxin genes, and deliberate release to the environment) appear in a new Section III-A and would require the same RAC review and NIH approval before initiation that currently hold for the prohibitions.

Current Guideline Section	Topic	Current Guidelines	RAC Proposal (Fed. Reg. 12/4/81)	Gottesman Proposal (Fed. Reg. 12/7/81)
I-E	Exemptions	Five classes of experiments exempt from the Guidelines.	Unchanged from current Guidelines.	Substance unchanged from current Guidelines. Moved to a new Section III-D.
III	Containment Levels for Covered Experiments	Experiments not otherwise prohibited or exempt are assigned a containment level (P1 to P3). All require prior IBC approval. Some require prior NIH approval. This Part of the Guidelines is very long and complex, classifying experiments into many groups and assigning a specific containment level to each.	*No prior IBC or NIH approval required. The entire current Part III of the Guidelines is replaced by an introduction, the two admonishments noted above and the following: "Where recommended physical containment levels applicable to non-recombinant DNA experiments exist for either the host or the vector, recombinant DNA experiments should be carried out at containment levels at least as high as those recommended for non-recombinant DNA experiments. If there is clear evidence that the donor DNA will significantly change the pathogenicity of the host, the containment level appropriate to the anticipated change will be applied. Otherwise, all experiments may be carried out under conditions of P1 or P1-LS physical containment."	Part III of the Guidelines is greatly simplified. As noted above, new Section III-A contains three of the current prohibitions. New Section III-D contains the current exemptions. New Section III-B specifies experiments requiring IBC approval before initiation. They are assigned containment levels, P1 to P4. (Many experiments in this Section currently require prior NIH approval.) New Section III-C to include all experiments not included in new Sections III-A, III-B or III-D. These experiments require IBC notification simultaneously with initiation of the experiment and can be carried out at P1 containment. (Experiments in this Section currently require prior IBC approval and containment level of P1 to P3.)
II	Containment	Defines physical and biological containment.	Unchanged from current Guidelines.	Unchanged from current Guidelines except for deletion of reference to HV3 systems.
V	Footnotes and References	Footnotes and references.	Changes as necessary to reflect other changes in Guidelines.	Changes as necessary to reflect other changes in Guidelines.

Current Guideline Section	Topic	Current Guidelines	RAC Proposal (Fed. Reg. 12/4/81)	Gottesman Proposal (Fed. Reg. 12/7/81)
VI	Voluntary Compliance	Policy for voluntary compliance with Guidelines.	Eliminated except for those portions of Section VI-F relevant to the protection of proprietary information.	Unchanged from current Guidelines.
I-A	Purpose	"I-A. 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."	Sentence added at end, "Adherence to these standards by all laboratories using recombinant DNA is recommended."	Unchanged from current Guidelines.
I-B	Definition of Recombinant DNA Molecules	"I-B Definition of Recombinant DNA Molecules. In the context of these Guidelines, recombinant DNA molecules are defined as either (i) molecules which are constructed outside living cells by joining natural or snythetic 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 (i) above."	Unchanged from current Guidelines.	Paragraph added at end, "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 is not expressed in vivo as a polynucleotide or polypeptide product, it is exempt from the Guidelines."
I-C	General Applicability	"I-C. General Applicability. See Section IV-B."	Eliminated.	Unchanged from current Guidelines.
Appendices	Appendices	Appendix A through H.	Changes as necessary to reflect other changes in Guidelines.	Changes as necessary to reflect other changes in Guidelines.

federal register

Friday
December 4, 1981

Part II

Department of Health and Human Services

National Institutes of Health

Recombinant DNA Research; Proposed Revised Guidelines

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Recombinant DNA Research: Proposed Revised Guidelines

AGENCY: National Institutes of Health, PHS, DHHS.

ACTION: Notice of proposed revision of the NIH guidelines for research involving recombinant DNA molecules.

SUMMARY: This notice sets forth a proposed revision of the 1981 NIH Guidelines for Research Involving Recombinant DNA Molecules (46 FR 34462). Interested parties are invited to submit comments concerning this proposal. This proposal and comments on it will be considered by the NIH Recombinant DNA Advisory Committee (RAC) at its next meeting.

DATE: Comments must be received by February 2, 1982.

ADDRESS: Written comments and recommendations should be submitted to the Director, Office of Recombinant DNA Activities, Building 31, Room 4A52, National Institutes of Health, Bethesda, Maryland 20205. All comments received in timely response to this notice will be considered and will be available for public inspection in the above office on weekdays between the hours of 8:30 a.m. and 5:00 p.m.

FOR FURTHER INFORMATION CONTACT: Additional information can be obtained from Drs. Stanley Barban or Elizabeth Milewski, Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20205, (301) 496-6061.

SUPPLEMENTARY INFORMATION: I am today issuing for public comment proposed revised NIH Guidelines for Research Involving Recombinant DNA Molecules. This action is taken in accordance with Section IV-E-1-b-(1) of the NIH Guidelines. This announcement introduces the proposed revision, then gives the proposed revised Guidelines in their entirety, and then includes a series of annexes containing relevant background documents. These annexes are:

Annex A: Original proposal of Drs. David Baltimore and Allan Campbell.
Annex B: Documents prepared by Working Group on Revision of the Guidelines.
Annex C: Minority reports of working group members.
Annex D: Draft Minutes of relevant portion of September 10-11, 1981, RAC Meeting.
Annex E: Current NIH Guidelines.

The history of development of these proposed revised Guidelines is as follows. Drs. David Baltimore and Allan Campbell, RAC members, had proposed a major revision of the Guidelines (Baltimore-Campbell proposed, Annex A), which was considered by the RAC at its April 1981 meeting. At the April 1981 meeting, a Working Group on Revision of the Guidelines was established to review the Baltimore-Campbell proposal as well as other approaches which might lead to a major revision of the Guidelines. The Working Group met on June 1, 1981, and on July 9, 1981. The Working Group prepared a proposal for revising the Guidelines, a summary of its actions, and a document entitled "Evaluation of the Risks Associated with Recombinant DNA Research." These documents appear as Annex B. Two minority reports were prepared by several members of the Working Group (Annex C). The Working Group report and the minority reports were distributed to RAC members prior to the September 1981 meeting.

The RAC extensively discussed the Working Group's report and other approaches to revision of the Guidelines at its September 1981 meeting. Draft minutes of that discussion constitute Annex D. As indicated in those draft minutes, the RAC passed by a vote of 16 in favor, 3 opposed, with 1 abstention, the elements of its version of a proposed revision of the Guidelines to be published for public comment. Based upon this proposal, NIH staff prepared the proposed revised Guidelines which are published immediately following this introduction, for public comment. For comparison, the current NIH Guidelines are given in Annex E.

The proposed revised Guidelines and comments on them will be considered by the RAC at its next meeting.

The major features of the proposed revision are:

1. The Guidelines would cease to be mandatory and would become a voluntary code of standard practice. Requirements that institutions have an Institutional Biosafety Committee (IBC), that investigators obtain prior approval from the IBC before beginning certain experiments, that investigators obtain prior approval from NIH before beginning certain experiments, and the section of the Guidelines specifying that noncompliance with the Guidelines could lead to loss of NIH funds, would all be eliminated.

2. Section III of the Guidelines giving containment levels would be greatly simplified, and most experiments currently mandated at P2 or P3 containment would be recommended at P1.

3. The prohibitions section (I-D) of the Guidelines would be eliminated, although two of the previous prohibitions would be retained instead as admonishments.

Proposed Guidelines for Research Involving Recombinant DNA Molecules

October 1981.

Table of Contents

I. Scope of the Guidelines

I-A. *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. Adherence to those standards by all laboratories using recombinant DNA is recommended.

I-B. *Definition of Recombinant DNA Molecules.* In the context of these Guidelines, recombinant DNA molecules are defined as either (i) 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 (i) above.

I-C. [Deleted]

I-D. [Deleted]

I-E. *Exemptions.* The following recombinant DNA molecules are exempt from these Guidelines:

I-E-1. Those that are not in organisms or viruses. [5]

I-E-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.

I-E-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 a 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).

I-E-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. A list of such exchangers will be prepared and periodically revised by the Director, NIH, with advice of the RAC, after appropriate notice and opportunity for public comment. (See Section IV-E-1-b-(1)-(d).) Certain classes are exempt as of publication of these Revised Guidelines. The list is in Appendix A. An updated list may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20205.

I-E-5. Other classes of recombinant DNA molecules, if the Director, NIH, 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. (See Section IV-E-1-b-(1)-(d).) Certain classes are exempt as of publication of these Revised Guidelines. The list is in Appendix C. An updated list may be obtained from the Office of Recombinant DNA Activities; National Institutes of Health, Bethesda, Maryland 20205.

I-F. *General Definitions.* See Section IV-C.

II. 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. (6-19) 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 varing degrees according to the estimated biohazard.

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 vestors 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 means of containment are complementary, different levels of containment appropriate for experiments with different recombinants can be established by apply various combinations of the physical and biological barriers along with a constant use of the standard practices. We consider these categories of containment separately here in order that such combinations can be conveniently expressed in the Guidelines.

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 recommend changes in the Guidelines to permit their use.

II-A *Standard Practices and Training.* The first principle of containment is a strict adherence to good microbiological practices. (6-15) Consequently, all personnel directly or indirectly involved in experiments on recombinant DNAs should receive adequate instruction. This should, 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 should have an emergency plan which describes the procedures to be followed if an accident contaminates personnel or the environment. The principal investigator should 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 where there is an effective vaccine it should be made available to all workers. Where serological monitoring is clearly appropriate it should be provided.

II-B *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 aganist 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 P1, P2, P3, and P4, 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. For example, the "Classification of Etiologic Agents on the Basis of Hazard,"[7] prepared by the Centers for Disease Control, describes four general levels which roughly correspond to our descriptions for P1, P2, P3, and P4; and the National Cancer Institute describes three levels for research on oncogenic viruses which roughly correspond to our P2, P3, and P4 levels.[8]

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 P3 and P4 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. Consideration will also be given by the Director, NIH, with the advice of the Recombinant DNA Advisory Committee to other combinations which achieve an equivalent level of containment. (See Section IV-E-1-b-(2)-(b).)

Information on large-scale applications can be found in "Physical Containment Recommendations for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules." **Federal Register,** April 11, 1980, where definitions are found of P1-LS, P2-LS, and P3-LS. Guidance is available from ORDA on physical containment levels when working with plant host-vector systems.

II-B-1. *P1 Level.*

II-B-1-a. *Laboratory Practices.*

II-B-1-a-(1). Laboratory doors shall be kept closed while experiments are in progress.

II-B-1-a-(2). Work surfaces shall be decontaminated daily, and immediately following spills of organisms containing recombinant DNA molecules.

II-B-1-a-(3). All biological wastes shall be decontaminated before disposal. Other contaminated materials, such as glassware, animal cages, and laboratory equipment, shall be decontaminated before washing, reuse, or disposal.

II-B-1-a-(4). Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

II-B-1-a-(5). Eating, drinking, smoking, and storage of foods are not permitted in the laboratory area in which recombinant DNA materials are handled.

II-B-1-a-(6). Persons shall wash their hands after handling organisms containing recombinant DNA molecules and when they leave the laboratory.

II-B-1-a-(7). Care shall be taken in the conduct of all procedures to minimize the creation of aerosols.

II-B-1-a-(8). Contaminated materials that are to be decontaminated at a site away from the laboratory shall be placed in a durable leak-proof container, which is closed before removal from the laboratory.

II-B-1-a-(9). An insect and rodent control program shall be instituted.

II-B-1-a-(10). The use of laboratory gowns, coats, or uniforms is discretionary with the laboratory supervisor.

II-B-1-a-(11). Use of the hupodermic needle and syringe shall be avoided when alternative methods are available.

II-B-1-a-(12). The laboratory shall be kept neat and clean.

II-B-1-b. *Containment Equipment.* Special containment equipment is not required at the P1 level.

II-B-1-c. *Special Laboratory Design.* Special laboratory design is not required at the P1 level.

II-B-2. *P2 Level.*

II-B-2-a. *Laboratory Practices.*

II-B-2-a-(1). Laboratory doors shall be kept closed while experiments are in progress.

II-B-2-a(2). Work surfaces shall be decontaminated daily, and immediately following spills of organisms containing recombinant DNA molecules.

II-B-2-a(3). All laboratory wastes shall be steam-sterilized (autoclaved) before disposal. Other contaiminated materials such as glassware, animal cages, laboratory equipment, and radiocative wastes shall be decontaminated by a means demonstrated to be effective before washing, reuse, or disposal.

II-B-2-a(4). Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

II-B-2-a(5). Eating, drinking, smoking, and storage of food are not permitted in the laboratory area in which recombinant DNA materials are handled.

II-B-2-a(6). Persons shall wash their hands after handling organisms containing recombinant DNA molecules and when they leave the laboratory.

II-B-2-a(7). Care shall be exercised to minimize the creation of aerosols. For example, manipulations such as inserting a hot inoculating loop or needle into a culture, flaming an inoculation loop or needle so that it splatters, and forceful ejection of fluids from pipettes or syringes shall be avoided.

II-B-2-a(8). Contaminated materials that are to be steam sterilized (autoclaved) or decontaminated at a site away from the laboratory shall be placed in a durable leak-proof container, which is closed before removal from the laboratory.

II-B-2-a(9). Only persons who have been advised of the nature of the research being conducted shall enter the laboratory.

II-B-2-a(10). The universal biohazard sign shall be posted on all laboratory access doors when experiments requiring P2 containment are in progress. Freezers and refrigerators or other units used to store organisms containing recombinant-DNA molecules shall also be posted with the universal biohazard sign.

II-B-2-a(11). An insect and rodent control program shall be instituted.

II-B-2-a(12). The use of laboratory gowns, coats, or uniforms is required. Laboratory clothing shall not be worn to the lunch room or outside of the building in which the laboratory is located.

II-B-2-a(13). Animals not related to the experiment shall not be permitted in the laboratory.

II-B-2-a(14). Use of the hypodermic needle and syringe shall be avoided when alternative methods are available.

II-B-2-a(15). The laboratory shall be kept neat and clean.

II-B-2-a(16). Experiments of lesser biohazard potential can be carried out concurrently in carefully demarcated areas of the same laboratory.

II-B-2-b. *Containment Equipment.* Biological safety cabinets [20] shall be used to contain aerosol-producing equipment, such as blenders, lyophilizers, sonicators, and centrifuges, when used to process organisms containing recombinant DNA molecules, except where equipment design provides for containment of the potential aerosol. For example, a centrifuge may be operated in the open if a sealed head or safety centrifuge cups are used.

II-B-2-c. *Special Laboratory Design.* An autoclave for sterilization of wastes and contaminated materials shall be

available in the same building in which organisms containing recombinant DNA molecules are used.

II–B–3. *P3 Level.*

II–B–3–a. *Laboratory Practices.*

II–B–3–a–(1). Laboratory doors shall be kept closed while experiments are in progress.

II–B–3–a–(2). Work surfaces shall be decontaminated following the completion of the experimental activity, and immediately following spills of organisms containing recombinant DNA molecules.

II–B–3–a–(3). All laboratory wastes shall be steam-sterilized (autoclaved) before disposal. Other contaminated materials, such as glassware, animal cages, laboratory equipment, and radioactive wastes, shall be decontaminated by a method demonstrated to be effective before washing, reuse, or disposal.

II–B–3–a–(4). Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

II–B–3–a–(5). Eating, drinking, smoking, and storage of food are not permitted in the laboratory area in which recombinant DNA materials are handled.

II–B–3–a–(6). Persons shall wash their hands after handling organisms containing recombinant DNA molecules and when they leave the laboratory.

II–B–3–a–(7). Care shall be exercised to minimize the creation of aerosols. For example, manipulations such as inserting a hot inoculating loop or needle into a culture, flaming an inoculation loop or needle so that it splatters, and forceful ejection of fluids from pipettes or syringes shall be avoided.

II–B–3–a–(8). Contaminated materials that are to be steam-sterilized (autoclaved) or decontaminated at a site away from the laboratory shall be placed in a durable leak-proof container, which is closed before removal from the laboratory.

II–B–3–a–(9). Entry into the laboratory shall be through a controlled access area. Only persons who have been advised of the nature of the research being conducted shall enter the controlled access area. Only persons required on the basis of program or support needs shall be authorized to enter the laboratory. Such persons shall be advised of the nature of the research being conducted before entry, and shall comply with all required entry and exit procedures.

II–B–3–a–(10). Persons under 16 years of age shall not enter the laboratory.

II–B–3–a–(11). The universal biohazard sign shall be posted on the controlled access area door and on all laboratory doors when experiments requiring P3-level containment are in progress. Freezers and refrigerators or other units used to store organisms containing recombinant DNA molecules shall also be posted with the universal biohazard sign.

II–B–3–a–(12). An insect and rodent control program shall be instituted.

II–B–3–a–(13). Laboratory clothing that protects street clothing (e.g., long-sleeve solid-front or wrap-around gowns, no-button or slipover jackets) shall be worn in the laboratory. Front-button laboratory coats are unsuitable. Laboratory clothing shall not be worn outside the laboratory and shall be decontaminated before it is sent to the laundry.

II–B–3–a–(14). Raincoats, overcoats, topcoats, coats, hats, caps, and such street outer-wear shall not be kept in the laboratory.

II–B–3–a–(15). Gloves shall be worn when handling materials requiring P3 containment. They shall be removed aseptically immediately after the handling procedure and decontaminated.

II–B–3–a–(16). Animals and plants not related to the experiment shall not be permitted in the laboratory.

II–B–3–a–(17). Vacuum outlets shall be protected by filter and liquid disinfectant traps.

II–B–3–a–(18). Use of hypodermic needle and syringe shall be avoided when alternative methods are available.

II–B–3–a–(19). The laboratory shall be kept neat and clean.

II–B–3–a–(20). If experiments involving other organisms which require lower levels of containment are to be conducted in the same laboratory concurrently with experiments requiring P3-level physical containment, they shall be conducted in accordance with all P3-level laboratory practices.

II–B–3–b. *Containment Equipment.*

II–B–3–b–(1). Biological safety cabinets[20] shall be used for all equipment and manipulations that produce aerosols—e.g., pipetting, dilutions, transfer operations, plating, flaming, grinding, blending, drying, sonicating, shaking, centrifuging—where these procedures involve organisms containing recombinant DNA molecules, except where equipment design provides for containment of the potential aerosol.

II–B–3–b–(2). Laboratory animals held in a P3 area shall be housed in partial-containment caging systems, such as Horsfall units[19A], open cages placed in ventilated enclosures, solid-wall and -bottom cages covered by filter bonnets, or solid-wall and -bottom cages placed on holding racks equipped with ultraviolet radiation lamps and reflectors.

Note.—Conventional caging systems may be used. *Provided,* That all personnel wear appropriate personal protective devices. These shall include, at a minimum, wrap-around gowns, head covers, gloves, shoe covers, and respirators. All personnel shall shower on exit from areas where these devices are required.

II–B–3–b–(3). *Alternative Selection of Containment Equipment.* Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment can be conducted in the P3 laboratory using containment equipment specified for the P2 level of physical containment. Experimental procedures involving a host-vector system that provides a one-step lower level of biological containment can be conducted in the P3 laboratory using containment equipment specified for the P4 level of physical containment. Alternative combinations of containment safeguards are shown in Table I.

TABLE I.—COMBINATIONS OF CONTAINMENT SAFEGUARDS

Classification of experiment		Alternate combinations of physical and biological containment			
		Physical Containment			
Physical containment	Biological[1] containment	Laboratory design specified for—	Laboratory practices specified for—	Containment equipment specified for—	Biological containment
P3	HV3	P3	P3	P3	HV3.
P3	HV3	P3	P3	P4	HV2.
P3	HV2	P3	P3	P3	HV2.
P3	HV2	P3	P3	P2	HV3.
P3	HV2	P3	P3	P4	HV1.
P3	HV1	P3	P3	P3	HV1.
P3	HV1	P3	P3	P2	HV2.

[1] See Section II-D for description of biological containment.

II–B–3–c. *Special Laboratory Design.*

II–B–3–c(1). The laboratory shall be separated by a controlled access area from areas that are open to unrestricted traffic flow. A controlled access area is an anteroom, a change room, an air lock or any other double-door arrangement

that separates the laboratory from areas open to unrestricted traffic flow.

II–B–3–c–(2). The surfaces of walls, floors, and ceilings shall be readily cleanable. Penetrations through these surfaces shall be sealed or capable of being sealed to facilitate space decontamination.

II–B–3–c–(3). A foot-, elbow-, or automatically-operated hand-washing facility shall be provided near each primary laboratory exit area.

II–B–3–c–(4). Windows in the laboratory shall be sealed.

II–B–3–c–(5). An autoclave for sterilization of wastes and contaminated materials shall be available in the same building (and preferably within the controlled laboratory area) in which organisms containing recombinant DNA molecules are used.

II–B–3–c–(6). The laboratory shall have a ventilation system that is capable of controlling air movement. The movement of air shall be from areas of lower contamination potential to areas of higher contamination potential (i.e., from the controlled access area to the laboratory area). If the ventilation system provides positive pressure supply air, the system shall operate in a manner that prevents the reversal of the direction of air movement or shall be equipped with an alarm that would actuated in the event that reversal in the direction of air movement were to occur. The exhaust air from the laboratory area shall not be recirculated to other areas of the building unless the exhaust air is filtered by HEPA filters or equivalent. The exhaust air from the laboratory area can be discharged to the outdoors without filtration or other means for effectively reducing an accidental aerosol burden provided that it can be dispersed clear of occupied buildings and air intakes.

II–B–3–c–(7). The treated exhaust-air from Class I and Class II biological safety cabinets [20] may be discharged either to the laboratory or to the outdoors. The treated exhaust-air from a Class III cabinet shall be discharged directly to the outdoors. If the treated exhaust-air from these cabinets is to be discharged to the outdoors through a building exhaust air system, it shall be connected to this system so as to avoid any interference with the air balance of the cabinet and the building ventilation system.

II–B–4. *P4 Level.*

II–B–4–a. *Laboratory Practices.*

II–B–4–a–(1). Laboratory doors shall be kept closed while experiments are in progress.

II–B–4–a–(2). Work surfaces shall be decontaminated following the completion of the experimental activity and immediately following spills of organisms containing recombinant DNA molecules.

II–B–4–a–(3). All laboratory wastes shall be steam-sterilized (autoclaved) before disposal. Other contaminated materials such as glassware, animal cages, laboratory equipment, and radioactive wastes shall be decontaminated by a method demonstrated to be effective before washing, reuse, or disposal.

II–B–4–a–(4). Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

II–B–4–a–(5). Eating, drinking, smoking, and storage of food are not permitted in the P4 facility.

II–B–4–a–(6). Persons shall wash their hands after handling organisms containing recombinant DNA molecules and when they leave the laboratory.

II–B–4–a–(7). Care shall be exercised to minimize the creation of aerosols. For example, manipulations such as inserting a hot inoculating loop or needle into a culture, flaming an inoculation loop or needle so that it splatters, and forceful ejection of fluids from pipettes or syringes shall be avoided.

II–B–4–a–(8). Biological materials to be removed from the P4 facility in a viable or intact state shall be transferred to a nonbreakable sealed container, which is then removed from the P4 facility through a pass-through disinfectant dunk tank or fumigation chamber.

II–B–4–a–(9). No materials, except for biological materials that are to remain in a viable or intact state, shall be removed from the P4 facility unless they have been steam-sterilized (autoclaved) or decontaminated by a means demonstrated to be effective as they pass out of the P4 facility. All wastes and other materials as well as equipment not damaged by high temperature or steam shall be steam-sterilized in the double-door autoclave of the P4 facility. Other materials which may be damaged by temperature or steam shall be removed from the P4 facility through a pass-through fumigation chamber.

II–B–4–a–(10). Materials within the Class III cabinets shall be removed from the cabinet system only after being steam-sterilized in an attached double-door autoclave or after being contained in a nonbreakable sealed container, which is then passed through a disinfectant dunk tank or a fumigation chamber.

II–B–4–a–(11). Only persons whose entry into the P4 facility is required to meet program or support needs shall be authorized to enter. Before entering, such persons shall be advised of the nature of the research being conducted and shall be instructed as to the appropriate safeguards to ensure their safety. They shall comply with instructions and all other required procedures.

II–B–4–a–(12). Persons under 18 years of age shall not enter the P4 facility.

II–B–4–a–(13). Personnel shall enter into and exist from the P4 facility only through the clothing change and shower rooms. Personnel shall shower at each egress from the P4 facility. Air locks shall not be used for personnel entry or exit except for emergencies.

II–B–4–a–(14). Street clothing shall be removed in the outer side of the clothing-change area and kept there. Complete laboratory clothing, including undergarments, head cover, shoes, and either pants and shirts or jumpsuits, shall be used by all persons who enter the P4 facility. Upon exit, personnel shall store this clothing in lockers provided for this purpose or discard it into collection hampers before entering the shower area.

II–B–4–a–(15). The universal biohazard sign is required on the P4 facility access doors and on all interior doors to individual laboratory rooms where experiments are conducted. The sign shall also be posted on freezers, refrigerators, or other units used to store organisms containing recombinant DNA molecules.

II–B–4–a–(16). An insect and rodent control program shall be instituted.

II–B–4–a–(17). Animals and plants not related to the experiment shall not be permitted in the laboratory in which the experiment is being conducted.

II–B–4–a–(18). Vacuum outlets shall be protected by filter and liquid disinfectant traps.

II–B–4–a–(19). Use of the hypodermic needle and syringe shall be avoided when alternate methods are available.

II–B–4–a–(20). The laboratory shall be kept neat and clean.

II–B–4–a–(21). If experiments involving other organisms which require lower levels of containment are to be conducted in the P4 facility concurrently with experiments requiring P4–level containment, they shall be conducted in accordance with all P4–level laboratory practices specified in this section.

II–B–4–b. *Containment Equipment.*

II–B–4–b–(1). Experimental procedures involving organisms that require P4–level physical containment shall be conducted either in (i) a Class III cabinet system or in (ii) Class I or Class II cabinets that are located in a specially designed area in which all personnel are

required to wear one-piece positive-pressure isolation suits.

II–B–4–b–(2). Laboratory animals involved in experiments requiring P4-level physical containment shall be housed either in cages contained in Class III cabinets or in partial containment caging systems (such as Horsfall units[19A], open cages placed in ventilated enclosures, or solid-wall and -bottom cages covered by filter bonnets, 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.

II–B–4–b–(3). *Alternative Selection of Containment Equipment.* Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment can be conducted in the P4 facility using containment equipment requirements specified for the P3 level of physical containment. Alternative combinations of containment safeguards are shown in Table II.

TABLE II.—COMBINATIONS OF CONTAINMENT SAFEGUARDS

Classification of experiment		Alternate combinations of physical and biological containment			
		Physical containment			
Physical containment	Biological [1] containment	Laboratory design specified for—	Laboratory practices specified for—	Containment equipment specified for—	Biological containment
P4	HV1	P4	P4	P4	HV1.
P4	HV1	P4	P4 [2]	P3	HV2.

[1] See Section II–D for description of biological containment.

[2] In this case gloves shall be worn, in addition to the clothing requirements specified in II–B–4–a–(14).

II–B–4–c. *Special Laboratory Design.*

II–B–4–c–(1). The laboratory shall be located in a restricted-access facility which is either a separate building or a clearly demarcated and isolated zone within a building. Clothing-change areas and shower rooms shall be provided for personnel entry and egress. These rooms shall be arranged so that personnel leave through the shower area to the change room. A double-door ventilated vestibule or ultraviolet air lock shall be provided for passage of materials, supplies, and equipment which are not brought into the P4 facility through the change room area.

II–B–4–c–(2). Walls, floors, and ceilings of the P4 facility are constructed to form an internal shell which readily allows vapor-phase decontamination and is animal- and insect-proof. All penetrations through these structures and surfaces are sealed. (The integrity of the walls, floors, ceilings, and penetration seals should ensure adequate containment of a vapor-phase decontaminant under static pressure conditions. This requirement does not imply that these surfaces must be airtight.)

II–B–4–c–(3). A foot-, elbow-, or automatically-operated handwashing facility shall be provided near the door within each laboratory in which experiments involving recombinant DNA are conducted in openface biological safety cabinets.

II–B–4–c–(4). Central vacuum systems are permitted. The system, if provided, shall not serve areas outside the P4 facility. The vacuum system shall include in-line HEPA filters near each use point or service cock. The filters shall be installed so as to permit in-place decontamination and replacement. Water supply, liquid and gaseous services provided to the P4 facility shall be protected by devices that prevent backflow.

II–B–4–c–(5). Drinking water fountains shall not be installed in laboratory or animal rooms of the P4 facility. Foot-operated water fountains are permitted in the corridors of the P4 facility. The water service provided to such fountains shall be protected from the water services to the laboratory areas of the P4 facility.

II–B–4–c–(6). Laboratory doors shall be self-closing.

II–B–4–c–(7). A double-door autoclave shall be provided for sterilization of material passing out of the P4 facility. The autoclave doors shall be interlocked so that both doors will not be open at the same time.

II–B–4–c–(8). A pass-through dunk tank or fumigation chamber shall be provided for removal from the P4 facility of material and equipment that cannot be heat-sterilized.

II–B–4–c–(9). All liquid effluents from the P4 facility shall be collected and decontaminated before disposal. Liquid effluents from biological safety cabinets and laboratory sinks shall be sterilized by heat. Liquid effluents from the shower and hand washing facilities may be activated by chemical treatment. HEPA filters shall be installed in all vents from effluent drains.

II–B–4–c–(10). An individual supply and exhaust-air ventilation system shall be provided. The system shall maintain pressure differentials and directional air flow as required to ensure inflow from areas outside the facility toward areas of highest potential risk within the facility. The system shall be designed to prevent the reversal of air flow. The system shall sound an alarm in the event of system malfunction.

II–B–4–c–(11). Air within individual laboratories of the P4 facility may be recirculated in HEPA filtered.

II–B–4–c–(12). The exhaust air from the P4 facility shall be HEPA filtered and discharged to the outdoors so that it is dispersed clear of occupied buildings and air intakes. The filter chambers shall be designed to allow *in situ* decontamination before removal and to facilitate certification testing after replacement.

II–B–4–c–(13). The treated exhaust-air from Class I and Class II biological safety cabinets[20] may be discharged directly to the laboratory room environment or to the outdoors. The treated exhaust-air from Class III cabinets shall be discharged to the outdoors. If the treated exhaust-air from these cabinets is to be discharged to the outdoors through the P4 facility exhaust air system, it shall be connected to this system so as to avoid any interference with the air balance of the cabinets or the facility exhaust air system.

II–B–4–c–(14). As noted in Section II–B–4–b–(1), the P4 facility may contain specially designed areas in which all personnel are required to wear one-piece positive-pressure isolation suits. Such areas shall be airtight. The exhaust-air from the suit area shall be filtered by two sets of HEPA filters installed in series, and a duplicate filtration unit and exhaust fan shall be provided. The air pressure within the suit area shall be less than that in any adjacent area. An emergency lighting system, communication systems, and power source shall be provided. A double-door autoclave shall be provided for sterilization of all waste materials to be removed from the suit area.

Personnel who enter this area shall wear a one-piece positive-pressure suit that is ventilated by a life-support system. The life-support system shall be provided with alarms and emergency backup air. Entry to this area is through an airlock fitted with airtight doors. A chemical shower area shall be provided to decontaminate the surfaces of the suit before removal.

II–C. *Shipment.* Recombinant DNA molecules contained in an organism or virus should be shipped only as an etiologic agent under requirements of the U.S. Public Health Service, and the U.S. Department of Transportation (§ 72.3, Part 72, Title 42, and §§ 173.386–173.388, Part 173, Title 49, U.S. Code of Federal Regulations (CFR)) as specified below:

II–C–1. Recombinant DNA molecules contained in an organism or virus

requiring P1, P2, or P3 physical containment, when offered for transportation or transported, should be subject to all requirements of § 72.3(a)–(e), Part 72, Title 42 CFR, and §§ 173.386–173.388, Part 173, Title 49 CFR.

II–C–2. Recombinant DNA molecules contained in an organism or virus requiring P4 physical containment, when offered for transportation or transported, should be subject to the requirements listed above under II–C–1 and are also subject to § 72.3(f), Part 72, Title 42 CFR.

II–C–3. Additional information on packaging and shipment is given in the "Laboratory Safety Monograph—A Supplement to the NIH Guidelines for Recombinant DNA Research."

II–D. *Biological Containment.*

II–D–1. *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 *H*ost-*V*ector, systems) for prokaryotes will be established; specific criteria will depend on the organisms to be used.

II–D–1–a. *HV1.* A host-vector system which provides a moderate level of containment. *Specific systems:*

II–D–1–a–(1). *EK1.* The host is always *E. coli* K–12 or a derivative thereof, and the vectors include nonconjugative plasmids (e.g., pSC101, ColEl, or derivatives thereof [12–27]) and variants of bacteriophage, such as lamboda [28–33]. The *E. coli* K–12 hosts shall not contain conjugation-proficient plasmids, whether autonomous or integrated, or generalized transducing phages.

II–D–1–a–(2). *Other Prokaryotes.* Hosts and vectors shall be, at a minimum, comparable in containment to *E. coli* K–12 with a non conjugative plasmid or bacteriophage vector. The data to be considered and a mechanism for approval of such HVl systems are described below (Section II–D–2).

II–D–1–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 specified conditions. *Specific systems:*

II–D–1–b–(1). For EK2 host-vector systems in which the vector is a plasmid, no more than one in 10^8 host cells should be able to perpetuate a cloned DNA fragment under the specified nonpermissive laboratory conditions designed to represent the natural environment, either by survival of the original host or as a consequence of transmission of the cloned DNA fragment.

II–D–1–b–(2). For EK2 host-vector systems in which the vector is a phage, no more than one in 10^8 phage particles should be able to perpetuate a cloned DNA fragment under the specified nonpermissive laboratory conditions designed to represent the natural environment either (i) as a prophage (in the inserted or plasmid form) in the laboratory host used for phage propagation or (ii) by surviving in natural environments and transferring a cloned DNA fragment to other hosts (or their resident prophages).

II–D–1–c. *HV3.* These are host-vector systems in which:

II–D–1–c–(1). All HV2 criteria are met.

II–D–1–c–(2). The vector is dependent on its propagation host or is highly defective in mobilizability. Reversion to host-independence must be less than $1/10^8$ per vector genome per generation.

II–D–1–c–(3). No markers conferring resistance to antibiotics commonly used clinically or in agriculture are carried by the vector, unless expression of such markers is dependent on the propagating host or on unique laboratory-controlled conditions or is blocked by the inserted DNA.

II–D–1–c–(4). The specified containment shown by laboratory tests has been independently confirmed by specified tests in animals, including primates, and in other relevant environments.

II–D–1–c–(5). The relevant genotypic and phenotypic traits have been independently confirmed.

II–D–2. *Certification of Host-Vector Systems.*

II–D–2–a. *Responsibility.* HV1 systems other than *E. coli* K–12, and HV2 and HV3 host-vector systems, may not be designated as such until they have been certified by the Director, NIH. Application for certification of a host-vector system is made by written application to the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20205.

Host-vector systems that are proposed for certification will be reviewed by the National Institutes of Health (NIH) Recombinant DNA Advisory Committee (RAC). (See Section IV–E–1–b–(1)–(c).) This will first involve review of the data on construction, properties, and testing of the proposed host-vector system by a Working Group composed of one or more members of the RAC and other persons chosen because of their expertise in evaluating such data. The Committee will then evaluate the report of the Working Group and any other available information at a regular meeting. The Director, NIH, is responsible for certification after receiving the advice of the RAC. Minor modifications of existing certified host-vector systems, where the modifications are of minimal or no consequence to the properties relevant to containment may be certified by the Director, NIH, without review by the RAC. (See Section IV–E–1–b–(3)–(f).)

When new host-vector systems are certified, notice of the certification will be sent by the Office of Recombinant DNA Activities (ORDA) to the applicant and to all Institutional Biosafety Committees (IBCs) and will be published in the *Recombinant DNA Technical Bulletin.* Copies of a list of all currently certified host-vector systems may be obtained from ORDA at any time.

The Director, NIH, may at any time rescind the certification of any host-vector system. (See Section IV–E–1–b–(3)–(g).) If certification of a host-vector system is rescinded, NIH will instruct investigators to transfer cloned DNA into a different system, or use the clones at a higher physical containment level unless NIH determines that the already constructed clones incorporate adequate biological containment.

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

II–D–2–b. *Data To Be Submitted for Certification.*

II–D–2–b–(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.

II–D–2–b–(2). *HV2 Systems.* Investigators planning to request HV2 certification for host-vector systems can obtain instructions from ORDA concerning data to be submitted (33A, 33B). 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 on 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. (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 (e.g., rodents). Such *in vivo* data may be required to confirm the validity of predicting *in vivo* survival on the basis of *in vitro* experiments.

Data must be submitted in writing to ORDA. Ten to twelve weeks are normally required for review and circulation of the data prior to the meeting at which such data can be considered by the RAC. Investigators are encouraged to publish their data on the construction, properties, and testing of proposed HV2 systems prior to consideration of the system by the RAC and its subcommittee. More specific instructions concerning the type of data to be submitted to NIH for proposed EK2 systems involving either plasmids or bacteriophage in *E. coli* K–12 are available from ORDA.

II–D–2–b–(3). *HV3 Systems.* Putative HV3 systems must, as the first step in certification, be certified as HV2 systems. Systems which meet the criteria given above under II–D–1–(c)–1, II–D–1–(c)–2, and II–D–1–(c)–3 will then be recommended for HV3 testing. Tests to evaluate various HV2 host-vector systems for HV3 certification will be performed by contractors selectd by NIH. These contractors will repeat tests performed by individuals proposing the HV2 system and, in addition, will conduct more extensive tests on conditions likely to be encountered in nature. The genotypic and phenotypic traits of HV2 systems will be evaluated. Tests on survival and transmissibility in and on animals, including primates, will be performed, as well as tests on survival in certain specified natural environments.

II–D–3. *Distribution of Certified Host-Vectors.* Certified HV2 and HV3 host-vector systems (plus appropriate control strains) must be obtained from the NIH or its designees, one of whom will be the investigator who developed the system. NIH shall announce the availability of the system by publication of notices in appropriate journals.

Plasmid vectors will be provided in a suitable host strain, and phage vectors will be distributed as small-volume lysates. If NIH propagates any of the host strains or phage, a sample will be sent to the investigator who developed the system or to an appropriate contractor, prior to distribution, for verification that the material is free from contamination and unchanged in phenotypic properties.

In distributing the certified HV2 and HV3 host-vector systems, NIH or its designee will (i) send out a complete description of the system; (ii) enumerate and describe the tests to be performed by the user in order to verify important phenotypic traits; (iii) remind the user that any modification of the system necessitates independent approval of the system by the NIH; and (iv) remind the user of responsibility for notifying ORDA of any discrepancies with the reported properties or any problems in the safe use of the system.

NIH may also distribute certified HV1 host-vector systems.

III. Containment Guidelines for Covered Experiments

Part III discusses experiments covered by the Guidelines. The reader should first consult Part I, where exempt experiments are listed.

Where recommended physical containment levels applicable to non-recombinant DNA experiments exist for either the host or the vector,* recombinant DNA experiments should be carried out at containment levels at least as high as those recommended for non-recombinant DNA experiments. If there is clear evidence that the donor DNA will significantly change the pathogenicity of the host, the containment level appropriate to the anticipated change will be applied. Otherwise, all experiments may be carried out under conditions of P1 or P1–LS physical containment.

No experiments should be performed which involve:

(a) 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 a drug to control disease agents in human or veterinary medicine or agriculture.

(b) Deliberate formation of recombinant DNAs containing genes for the biosynthesis of toxins lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight (e.g., the botulinum toxins, tetanus toxin, diphtheria toxin, *Shigella dysenteriae* neurotoxin). Guidelines for the cloning of DNAs containing genes coding for the biosynthesis of toxins which are lethal to vertebrates at 100 nanograms to 100 micrograms per kilogram body weight are specified in Appendix G.

IV. Roles and Responsibilities

IV–A. [Deleted]

IV–B. [Deleted]

IV–C. *General Definitions.* The following terms are defined as follows:

IV–C–1. "DNA" means deoxyribonucleic acid.

IV–C–2. "Recombinant DNA" or "recombinant DNA molecules" means either (i) 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 which result from the replication of a molecule described in (i) above.

IV–C–3. [Deleted]

IV–C–4. "Institution" means any public or private entity (including Federal, State, and local government agencies).

IV–C–5. [Deleted]

IV–C–6. "NIH Office of Recombinant DNA Activities" or "ORDA" means the office within NIH with responsibility for

* Such as those specified by the CDC Guidelines, or the USDA Quarantine Regulations.

(i) reviewing and coordinating all activities of NIH related to the Guidelines, and (ii) performing other duties as defined in Section IV–E–3.

IV–C–7. "Recombinant DNA Advisory Committee" or "RAC" means the public advisory committee that advises the Secretary, the Assistant Secretary for Health, and the Director of the National Institutes of Health concerning recombinant DNA research. The RAC shall be constituted as specified in Section IV–E–2.

IV–C–8. "Director, NIH" or "Director" means the Director of the National Institutes of Health and any other officer or employee of NIH to whom authority has been delegated.

IV–C–9. [Deleted]

IV–C–10. [Deleted]

IV–C–11. "Laboratory Safety Monograph" or "LSM" means a publication to accompany the NIH Guidelines describing practices, equipment, and facilities in detail.

IV–D. *Responsibilities of the Institution.*

Each institution conducting or sponsoring recombinant DNA research should take responsibility for monitoring its own activities in this area. Any unusual events that might be associated with the use of recombinant DNA molecules should be reported to the Director, NIH.

IV–E. *Responsibilities of NIH.*

IV–E–1. *Director.* The Director, NIH, is responsible for (i) establishing the NIH Guidelines on recombinant DNA research, (ii) overseeing their implementation, and (iii) their final interpretation.

The Director has a number of responsibilities under the Guidelines that involve the NIH Office of Recombinant DNA Activities (ORDA) and the Recombinant DNA Advisory Committee (RAC). ORDA's responsibilities under the Guidelines are administrative. Advice from the RAC is primarily scientific and technical. In certain circumstances, there is specific opportunity for public comment, with published response, before final action.

IV–E–1–a. *General Responsibilities of the Director, NIH.* The responsibilities of the Director shall include the following:

IV–E–1–a–(1). Promulgating requirements as necessary to implement the Guidelines;

IV–E–1–a–(2). Establishing and maintaining the RAC to carry out the responsibilities set forth in Section IV–E–2. The RAC's membership is specified in its charter and in Section IV–E–2; and

IV–E–1–a–(3). Establishing and maintaining ORDA to carry out the responsibilities defined in Section IV–E–3.

IV–E–1–b. *Specific Responsibilities of the Director, NIH.*

IV–E–1–b–(1). *The Director is responsible for the following major actions* (For these, the Director must seek the advice of the RAC and provide an opportunity for public and Federal agency comment. Specifically, the agenda of the RAC meeting citing the major actions will be published in the **Federal Register** at least 30 days before the meeting, and the Director will also publish the proposed actions in the **Federal Register** for comment at least 30 days before the meeting. In addition, the Director's proposed decision, at his discretion, may be published in the **Federal Register** for 30 days of comment before final action is taken. The Director's final decision, along with response to the comments, will be published in the **Federal Register** and the *Recombinant DNA Technical Bulletin.* The RAC will be notified of this decision):

IV–E–1–b–(1)–(a). Changing containment levels for types of experiments that are specified in the Guidelines when a major action is involved;

IV–E–1–b–(1)–(b). Assigning containment levels for types of experiments that are not explicitly considered in the Guidelines when a major action is involved;

IV–E–1–b–(1)–(c). Certifying new host-vector systems, with the exception of minor modifications of already certified systems. (The standards and procedures for certification are described in Section II–D–2–a. Minor modifications constitute, for example, those of minimal or no consequence to the properties relevant to containment.);

IV–E–1–b–(1)–(d). Promulgating and amending a list of classes of recombinant DNA molecules to be exempt from these Guidelines because they consist entirely of DNA segments from species that exchange DNA by known physiological processes, or otherwise do not present a significant risk to health or the environment (see Sections I–E–4 and –5 for further information);

IV–E–1–b–(1)–(e). [Deleted]

IV–E–1–b–(1)–(f). Adopting other changes in the Guidelines.

IV–E–1–b–(2). *The Director is also responsible for the following lesser actions* (For these, the Director must seek the advice of the RAC. The Director's decision will be transmitted to the RAC and published in the *Recombinant DNA Technical Bulletin):*

IV–E–1–b–(2)–(a). Interpreting and determining containment levels, upon request by ORDA;

IV–E–1–b–(2)–(b). Changing containment levels for experiments that are specified in the Guidelines;

IV–E–1–b–(2)–(c). Assigning containment levels for experiments not explicitly considered in the Guidelines;

IV–E–1–b–(2)–(d). [Deleted]

IV–E–1–b–(2)–(e). [Deleted]

IV–E–1–b–(3). *The Director is also responsible for the following actions.* (The Director's decision will be transmitted to the RAC and published in the *Recombinant DNA Technical Bulletin):*

IV–E–1–b–(3)–(a). Interpreting the Guidelines for experiments to which the Guidelines specifically assign containment levels;

IV–E–1–b–(3)–(b). Determining appropriate containment conditions for experiments according to case precedents developed under Section IV–E–1–b–(2)–(c).

IV–E–1–b–(3)–(c). [Deleted]

IV–E–1–b–(3)–(d). [Deleted]

IV–E–1–b–(3)–(e). [Deleted]

IV–E–1–b–(3)–(f). Approving minor modifications of already certified host-vector systems. (The standards and procedures for such modifications are described in Section II–D–2); and

IV–E–1–b–(3)–(g). Decertifying already certified host-vector systems.

IV–E–1–b–(3)–(h). [Deleted]

IV–E–1–b–(3)–(i). Adding new entries to the list of toxins for vertebrates (see Appendix G).

IV–E–1–b–(3)–(j). Approving the cloning of toxin genes in host-vector systems other than *E. coli* K–12 (see Appendix G).

IV–E–1–b–(4). [Deleted]

IV–E–1–b–(5). [Deleted]

IV–E–2. *Recombinant DNA Advisory Committee.* The NIH Recombinant DNA Advisory Committee (RAC) is responsible for carrying out specified functions cited below as well as others assigned under its charter or by the Secretary, HHS, the Assistant Secretary for Health, and the Director, NIH.

The members of the committee shall be chosen to provide, collectively, expertise in scientific fields relevant to recombinant DNA technology and biological safety—e.g., microbiology, molecular biology, virology, genetics, epidemiology, infectious diseases, the biology of enteric organisms, botany, plant pathology, ecology, and tissue culture. At least 20 percent of the members shall be persons knowledgeable in applicable law, standards of professional conduct and practice, public attitudes, the

environment, public health, occupational health, or related fields. Representatives from Federal agencies shall serve as nonvoting members. Nominations for the RAC may be submitted to the NIH Office of Recombinant DNA Activities, Bethesda, Md. 20205.

All meetings of the RAC will be announced in the **Federal Register,** including tentative agenda items, 30 days in advance of the meeting, with final agendas (if modified) available at least 72 hours before the meeting. No item defined as a major action under Section IV–E–1–b–(1) may be added to an agenda after it appears in the **Federal Register.**

IV–E–2–a. *The RAC shall be responsible for advising the Director, NIH, on the actions listed in Section IV–E–1–b–(l) and –(2).*

IV–E–3. *The Office of Recombinant DNA Activities.* ORDA shall serve as a focal point for information on recombinant DNA activities and provide advice to all within and outside NIH, including Institutions, Principal Investigators, Federal agencies, State an local governments, and institutions in the private sector. ORDA shall carry out such other functions as may be delegated to it by the Director, NIH, including those authorities described in Section IV–E–1–b–(3). In addition, ORDA shall be responsible for the following:

IV–E–3–a. through IV–E–3–c–(3). [Deleted]

IV–E–3–c–(4). Publish in the **Federal Register** announcements of RAC meetings and agendas 30 days in advance on any action listed in Section IV–E–1–b–(1) and Section IV–E–1–b–(2).

Note.—If the agenda for an RAC meeting is modified, ORDA shall make the revised agenda available to anyone, upon request, at least 72 hours in advance of the meeting.

IV–E–3–c–(5). Publish the *Recombinant DNA Technical Bulletin;* and

IV–E–3–c–(6). Serve as executive secretary to the RAC.

IV–E–4. *Other NIH Components.* Other NIH components shall be responsible for:

IV–E–4–a. [Deleted]

IV–E–4–b. [Deleted]

IV–E–4–c. Announcing and distributing certified HV2 and HV3 host-vector systems (see Section II–E–3).

IV–F. [Deleted]

IV–G. [Deleted]

V. Footnotes and References

(1)–(4) [Deleted]

(5) Care should be taken to inactivate recombinant DNA before disposal. Procedures for inactivating DNA can be found in the "Laboratory Safety Monograph: A Supplement to the NIH Guidelines for Recombinant DNA Research."

(6) *Laboratory Safety at the Center for Disease Control* (Sept. 1974). U.S. Department of Health, Education and Welfare Publication No. CDC 75–8118.

(7) *Classification of Etiological Agents on the Basis of Hazard.* (4th Edition, July 1974). U.S. Department of Health, Education and Welfare. Public Health Service. Centers for Disease Control, Office of Biosafety, Atlanta, Georgia 30333.

(8) *National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses* (Oct. 1974). U.S. Department of Health, Education and Welfare Publication No. (NIH) 75–790.

(9) *National Institutes of Health Biohazards Safety Guide* (1974). U.S. Department of Health, Education, and Welfare, Public Health

(10) *Biohazards in Biological Research* (1973). A. Hellman, M. N. Oxman, and R. Pollack (ed.) Cold Spring Harbor Laboratory.

(11) *Handbook of Laboratory Safety* (1971). Second Edition. N. V Steere (ed.). The Chemical Rubber Co., Cleveland.

(12) Bodily, J. L. (1970). *General Administration of the Laboratory,* H. L. Bodily, E. L. Updyke, and J. O. Mason (eds.), Diagnostic Procedures for Bacterial Mycotic and Parasitic Infections. American Public Health Association, New York, pp. 11–28.

(13) Darlow, H. M. (1969). *Safety in the Microbiological Laboratory.* In J. R. Norris and D. W. Robbins (ed.), Methods in Microbiology. Academic Press, Inc. New York. pp. 169–204.

(14) *The Prevention of Laboratory Acquired Infection* (1974). C. H. Collins, E. G. Hartley, and R. Pilsworth. Public Health Laboratory Service, Monograph Series No. 6.

(15) Chatigny, M. A. (1961). *Protection Against Infection in the Microbiological Laboratory: Devices and Procedures.* In W. W. Umbreit (ed.). Advances in Applied Microbiology. Academic Press, New York, N.Y. 3:131–192.

(*16*) Design Criteria for Viral Oncology Research Facilities (1975). U.S. Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, DHEW Publication No. (NIH) 75–891.

(*17*) Kuehne, R. W. (1973. *Biological Containment Facility for Studying Infectious Disease.* Appl. Microbiol. 26–239–243.

(*18*) Runkle, R. S., and G. B. Phillips (1969). *Microbial Containment Control Facilities.* Van Nostrand Reinhold, New York.

(*19*) Chatigny, M. A., and D. I. Clinger (1969). *Contamination Control in Aerobiology.* In R. L. Dimmick and A. B. Akers (eds.). An Introduction to Experimental Aerobiology. John Wiley & Sons, New York, pp. 194–263.

(*19A*) Horsfall, F. L., Jr., and J. H. Baner (1940). *Individual Isolation of Infected Animals in a Single Room.* J. Bact. *40,* 569–580.

(*20*) Biological safety cabinets referred to in this section are classified as *Class I, Class II,* or *Class III* cabinets. A *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. A *Class II* cabinet 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. Design and performance specifications for *Class II* cabinets have been adopted by the National Sanitation Foundation, Ann Arbor, Michigan. A *Class III* cabinet 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.

(*21*) Hershfield, V., H. W Boyer, C. Yanofsky, M. A. Lovett, and D. R. Helinski (1974). *Plasmid ColEl as a Molecular Vehicle for Cloning and Amplification of DNA.* Proc. Nat. Acad. Sci. USA *71* 3455–3459.

(*22*) Wensink, P. C., D. J. Finnegan, J. E. Donelson, and D. S. Hogness (1974). *A System for Mapping DNA Sequences in the Chromosomes of Drosophila Melanogaster.* Cell *3,* 315–335.

(*23*) Tanaka, T., and B. Weisblum (1975). *Construction of a Colicin El–R Factor Composite Plasmid In Vitro: Means for Amplification of Deoxyribonucleic Acid.* J. Bacteriol. *121,* 354–362.

(*24*) Armstrong, K. A., V Hershfield, and D. R. Helinski (1977). *Gene Cloning and Containment Properties of Plasmid Col El and Its Derivatives,* Science *196,* 172–174.

(*25*) Bolivar, F., R. L. Rodriguez, M. C. Betlach, and H. W Boyer (1977). *Construction and Characterization of New Cloning Vehicles: I. Ampicillin-Resistant Derivative of pMB9.* Gene *2,* 75–93.

(*26*) Cohen, S. N., A. C. W Chang, H. Boyer, and R. Helling (1973). *Construction of Biologically Functional Bacterial Plasmids in Vitro.* Proc. Natl. Acad. Sci. USA *70,* 3240–3244.

(*27*) Bolivar, F., R. L. Rodriguez, R. J. Greene, M. C. Batlach, H. L. Reyneker, H. W. Boyer, J. H. Crosa, and S. Falkow (1977). *Construction and Characterization of New Cloning Vehicles: II A Multi-Purpose Cloning System.* Gene *2,* 95–113.

(*28*) Thomas, M., J. R. Cameron, and R. W. Davis (1974). *Viable Molecular Hybrids of Bacteriophage Lambda and Eukaryotic DNA.* Proc. Nat. Acad. Sci. USA *71,* 4579–4583.

(29) Murray, N. E., and K. Murray (1974). *Manipulation of Restriction Targets in Phage Lambda to Form Receptor Chromosomes for DNA Fragments.* Nature *251,* 476–481.

(30) Rambach, A., and P. Tiollais (1974). *Bacteriophage Having EcoRI Endonuclease Sites Only in the Non-Essential Region of the Genome.* Proc. Nat. Acad. Sci., USA *71,* 3927–3930.

(31) Blattner, F. R., B. G. Williams, A. E. Bleche, K. Denniston-Thompson, H. E. Faber, L. A. Furlong, D. J. Gunwald, D. O. Kiefer, D. D. Moore, J. W. Shumm, E. L. Sheldon, and O. Smithies (1977). *Charon Phages: Safer Derivatives of Bacteriophage Lambda for DNA Cloning.* Science *196,* 163–169.

(32) Donoghue, D. J., and P. A. Sharp (1977). *An Improved Lambda Vector: Construction of Model Recombinants Coding for Kanamycin Resistance,* Gene *1,* 209–227.

(33) Leder, P., D. Tiemeier and L. Enquist (1977). *EK2 Derivatives of Bacteriophage Lambda Useful in the Cloning of DNA from Higher Organisms: The gt WES System.* Science *196.* 175–177.

(33A) Skalka, A. (1978). *Current Status of Coliphage EK2 Vectors.* Gene *3,* 29–35.

(33B) Szybalski, W., A. Skalka, S. Gottesman, A. Campbell, and D. Botstein (1978). *Standardized Laboratory Tests for EK2 Certification.* Gene *3,* 36–38.

(34) [Deleted]

(35) Defined as observable under optimal laboratory conditions by transformation, transduction, phage infection, and/or conjugation with transfer of phage, plasmid, and/or chromosomal genetic information. Note that this definition of exchange may be less stringent than that applied to exempt organisms under Section I–E–4.

(36–48) [Deleted]

(49) A subset of non-conjugative plasmid vectors are also poorly mobilizable (e.g., pBR322, pBR313). Where practical, these vectors should be employed.

(50) [Deleted]

VI. Voluntary Compliance

VI–A. to VI–E. [Deleted]

VI–F. *Protection of Proprietary Data.* In general, the Freedom of Information Act requires Federal agencies to make thier records available to the public upon request. However, this requirement does not apply to, among other things, "trade secrets and commercial and financial information obtained from a person and privileged or confidential." 18 U.S.C. 1905, in turn, makes it a crime for an officer or employee of the United States or any Federal department or agency to publish, divulge, disclose, or make known "in any manner or to any extent not authorized by law any information coming to him in the course of his employment or official duties or by reason of any examination or investigation made by, or return, report or record made to or filed with, such department or agency or officer or employee thereof, which information concerns or relates to the trade secrets, (or processes * * * of any person, firm, partnership, corporation, or association." This provision applies to all employees of the Federal Government, including special Government employees. Members of the Recombinant DNA Advisory Committee are "special Government employees."

VI–F–1. In submitting information to NIH for purposes of complying voluntarily with the Guidelines, an institution may designate those items of information which the institution believes constitute trade secrets or privileged or confidential commercial or financial information.

VI–F–2. If NIH receives a request under the Freedom of Information Act for information so designated, NIH will promptly contact the institution to secure its views as to whether the information (or some portion) should be released.

VI–F–3. If the NIH decides to release this information (or some portion) in response to a Freedom of Information request or otherwise, the institution will be advised; and the actual release will not be made until the expiration of 15 days after the institution is so advised, except to the extent that earlier release, in the judgment of the Director, NIH, is necessary to protect against an imminent hazard to the public or the environment.

VI–F–4. The following information will usually be considered publicly available information, consistent with the need to protect proprietary data:

a. The names of the institution and principal investigator.

b. The location where the experiments will be performed.

c. The host-vector system.

d. The source of the DNA.

e. The level of physical containment.

VI–F–5–a. Any institution, which is considering submission of data or information vountarily to NIH, may request presubmission review of the records involved to determine whether, if the records are submitted, NIH will or will not make part or all of the records available upon request under the Freedom of Information Act.

VI–F–5–b. A request for presubmission review should be submitted to ORDA, along with the records involved. These records must be clearly marked as being the property of the institution, on loan to NIH solely for the purpose of making a determination under the Freedom of Information Act. ORDA will then seek a determination from the HEW Freedom of Information Officer, the responsible official under HEW regulations (45 CFR Part 5), as to whether the records involved (or some portion) are or are not available to members of the public under the Freedom of Information Act. Pending such a determination, the records will be kept separate from ORDA files, will be considered records of the institution and not ORDA, and will not be received as part of ORDA files. No copies will be made of the records.

VI–F–5–c. ORDA will inform the institution of the HEW Freedom of Information Officer's determination and follow the institution's instructions as to whether some or all of the records involved are to be returned to the institution or to become a part of ORDA files. If the institution instructs ORDA to return the records, no copies or summaries of the records will be made or retained by HEW, NIH, or ORDA.

VI–F–5–d. The HEW Freedom of Information Officer's determination will represent that official's judgment, as of the time of the determination, as to whether the records involved (or some portion) would be exempt from disclosure under the Freedom of Information Act, if at the time of the determination the records were in ORDA files and a request were received from them under the Act.

Appendix A.—Exemptions Under I–E–4

Section I–E–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. A list of such exchangers will be prepared and periodically revised by the Director, NIH, with advice of the Recombinant DNA Advisory Committee, after appropriate notice and opportunity for public comment (see Section IV–E–1–b–(1)–(d).) Certain classes are exempt as of publication of these Revised Guidelines. The list is in Appendix A."

Under exemption I–E–4 of these revised 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,* eighth edition. R.E. Buchanan and N.E. Gibbons, editors. Williams and Wilkins Company: Baltimore, 1974.)

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*

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

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*

Appendix B.—Classification of Microorganisms on the Basis of Hazard

I. Classification of Etiologic Agents on the Basis of Hazard (1)

A. Class 1 Agents

All bacterial, parasitic, fungal, viral, rickettsial, and chlamydial agents not included in higher classes.

B. Class 2 Agents

1. Bacterial Agents

Actinobacillus—all species except *A. mallei*, which is in Class 3
Arizona hinshawii—all serotypes
Bacillus anthracis
Bordetella—all species
Borrelia recurrentis, B. vincenti
Clostridium botulinum,
Cl. *chauvoei*, Cl. *haemolyticum,*
Cl. *histolyticum*, Cl. *novyi,*
Cl. *septicum*, Cl. *tetani*
Corynebacterium diptheriae,
C. *equi*, C. *haemolyticum*
C. *pseudotuberculosis*
C. *pyogenes, C. renale*
Diplococcoccus (Streptococcus) pneumoniae
Erysipelothrix insidiosa
Escherichia coli—all enteropathogenic serotypes
Haemophilus ducreyi, H. influenzae
Herellae vaginicola
Klebsiella—all species and all serotypes
Leptospira interrogans—all serotypes
Listeria—all species
Mima poloymorpha
Moraxella—all species
Mycobacteria—all species except those listed in Class 3
Mycoplasma—all species except *Mycoplasma mycoides* and *Mycoplasma agalactiae,* which are in Class 5
Neisseria gonorrhoeae, N. meningitidis
Pasteurella—all species except those listed in Class 3
Salmonella—all species and all serotypes
Shigella—all species and all serotypes
Sphaerophorus necrophorus
Staphylococcus aureaus
Streptobacillus moniliformis
Streptococcus pyogenes
Treponema carateum, T. pallidum, and T. pertenue
Vibrio fetus, V. comma, including biotype El Tor, and *V. parahemolyticus*

2. Fungal Agents

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

3. Parasitic Agents

Endomoeba histolytica
Leishmania sp.
Naegleria gruberi
Toxoplasma gondii
Toxocara canis
Trichinella spiralis
Trypanosoma cruzi

4. Viral, Rickettsial, and Chlamydial Agents

Adenoviruses—human—all types
Cache Valley virus
Coxsackie A and B viruses
Cytomegloviruses
Echoviruses—all types
Encephalomyocarditis virus (EMC)
Flanders virus
Hart Park virus
Hepatitis-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
Lymhogranuloma 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, Monkey pox, and Whitepox,* which depending on experiments, are in Class 3 or Class 4
Rabies virus—all strains except *Rabies street virus,* which should be classified in Class 3 when inoculated into carnivores
Reoviruses—all types
Respiratory syncytial virus
Rhinoviruses—all types
Rubella virus
Simian viruses—all types except *Herpesvirus simiae (Monkey B virus) and Marburg virus,* which are in Class 4
Sindbis virus
Tensaw virus
Turlock virus
Vaccinia virus
Varicella virus
Vole rickettsia
Yellow fever virus, 17D vaccine strain

C. Class 3 Agents

1. Bacterial Agents

*Actinobacillus mallei**
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 pseudomallei**
Yersenia pestis

2. Fungal Agents

Coccidioides immitis
Histoplasma capsulatum
Histoplasma capsulatum var. *duboisii*

3. Parasitic Agents

Schistosoma mansoni

4. Viral, Rickettsial, and Chlamydial Agents

***Alastrim, Smallpox, Monkey pox, and Whitepox,* when used *in vitro*
Arboviruses—all strains except those in Class 2 and 4 (Arboviruses indigenous to the United States are in Class 3, except those listed is Class 2.
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
Lumphocytic choriomeningitis virus (LCM)
Psittacosis-Ornithosis-Trachoma group of agents
Rabies street virus, when used in inoculations of carnivores (See Class 2)
Rickettsi—all species except *Vole rickettsia* when used for transmission or animal inoculation experiments
*Vesicular stomatitis virus**
Yellow fever virus—wild, when used *in vitro*

D. Class 4 Agents

1. Bacterial Agents

None

2. Fungal Agents

None

3. Parasitic Agents

None

4. Viral, Rickettsial, and Chlamydial Agents

***Alastrim, Smallpox, Monkey pox, and Whitepox,* 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 diesase, 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

II. Classification of Oncogenic Viruses on the Basis of Potential Hazard (2)

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

B. Moderate-Risk Oncogenic Viruses

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

III. Animal Pathogens (3)

A. Animal disease organisms which are forbidden entry into the United States by Law (CDC Class 5 agents)

1. Foot and mouth disease virus

B. Animal disease organisms and vectors which are forbidden entry into the United States by USDA Policy (CDC Class 5 Agents)

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 evansi
Theileria parva (East Coast fever)
Theileria annulata
Theileria lawrencei
Theileria bovis
Theileria hirci
Vesicular exanthema virus
Wesselsbron disease virus
Zyonema

Footnotes and References of Appendix B

*A USDA permit, required for import and interstate commerce of pathogens, may be obtained from the Animal and Plant Health Inspection Service, USDA, Federal Building, Hyattsville, MD. 20782.

**Since the publication of the classification in 1974 [1], the *Actinomycetes* have been reclassified as bacterial rather than fungal agents.

***All activities, including storage of variola and whitepox are restricted to the single national facility (World Health Organization (WHO) Collaborating Center for Smallpox Research, Center for Disease Control, in Atlanta).

(1) Classification of Etiologic 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.

(2) National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses. (October 1974). U.S. Department of Health, Education, and Welfare Publication No. (NIH) 75–790.

(3) U.S. Department of Agriculture, Animal and Plant Health Inspection Service.

Appendix C.—Exemptions Under I–E–5

Section I–E–5 states that exempt from these Guidelines are "Other classes of recombinant DNA molecules, if the Director, NIH, with advice of the Recombinant DNA Advisory Committee, after appropriate notice and opportunity for public comment, finds that they do not present a significant risk to health or the environment. (See Section IV–E–1–b–(1)–(d)). Certain classes are exempt as of publication of these Revised Guidelines."

The following classes of experiments are exempt under Section I–E–5 of the Guidelines:

1. *Recombinant DNAs in Tissue Culture.* Recombinant DNA molecules derived entirely from non-viral components (that is, no component is derived from a eukaryotic virus), that are propagated and maintained in cells in tissue culture are exempt from these Guidelines with the exceptions listed below.

Exceptions. Experiments, involving the deliberate introduction of genes coding for the biosynthesis of toxins potent for vertebrates. (See Appendix G.)

2. *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 (a) the *E. coli* host shall not contain conjugation proficient plasmids or generalized transducing phages, and (b) lambda or lambdoid or Ff bacteriophages or nonconjugative plasmids [49] shall be used as vectors. However, experiments involving the insertion into *E. coli* K–12 of DNA from prokaryotes that exchange genetic information [35] with *E. coli* may be performed with any *E. coli* K–12 vector (e.g., conjugative 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 experiments, P1 physical containment conditions are recommended.

Exceptions. Experiments involving the deliberate cloning of genes coding for the biosynthesis of toxins potent for vertebrates. (See Appendix G.)

3. *Experiments Involving Saccharomyces cerevisiae host-vector systems.* Experiments which use *Saccharomyces cerevisiae* host-vector systems, with the exception of experiments listed below, are exempt from these Guidelined provided that laboratory strains are used.

For these exempt experiments, P1 physical containment conditions are recommended.

Exceptions. Experiments involving the deliberate cloning of genes coding for the biosynthesis of toxins potent for vertebrates. (See Appendix G.)

4. *Experiments Involving Bacillus subtilis host-vector systems.* Any asporogenic *Bacillus subtilis* strain which does not revert to a sporeformer with a frequency greater than 10^{-7} can be used for cloning DNA from any nonprohibited source, with the exception of those experiments listed below. Indigenous *Bacillus* plasmids and phages, whose host-range does not include *Bacillus cereus* or *Bacillus anthracis,* may be used as vectors.

For these exempt experiments P1 physical containment conditions are recommended.

Exceptions. Experiments involving the deliberate cloning of genes coding for the biosynthesis of toxins potent for vertebrates. (See Appendix G.)

Appendix D [Deleted]

Appendix E [Deleted]

Appendix F.—Certified Host-Vector Systems

A listing of host-vector systems previously classified as HV1 or HV2 follows.

HV1—The following plasmids are accepted as the vector components of certified *B. subtilis* HV1 systems; pUB110, pC194, pS194, pSA2100, pE194, pT127, pUB112, pC221, pC223, and pAB124. *B. subtilis* strains RUB 331 and BGSC 1S53 have been certified as the host component of HV1 systems based on these plasmids.

HV1—The following specified strains of *Neurospora crassa* which have been modified to prevent aerial dispersion:

(1) inl (inositolless) strains 37102, 37401, 46316, 64001, and 89601.

(2) csp–1 strain UCLA and csp–2 strains FS 590, UCLA101 (these are conidial separation mutants).

(3) eas strain UCLA191 (an "easily wettable" mutant).

HV1—The following *Streptomyces* species: *Streptomyces coelicolor, S. lividans, S. parvulus,* and *S. griseus.* The following are accepted as vector components of certified *Streptomyces* HV1 systems: *Streptomyces* plasmids SCP2, SLP1.2, pIJ101, actinophage phi C31, and their derivatives.

HV2—The asporogenic mutant derivative of *Bacillus subtilis,* ASB 298, with the following plasmids as the vector component: pUB110, pC194, pS194, pSA2100, pE194, pT127, pUB112, pC221, pC223, and pAB124.

HV2—The following sterile strains of *Saccharomyces cerevisiae,* all of which have the ste–VC9 mutation, SHY1, SHY2, SHY3, and SHY4. The following plasmids are certified for use: YIp1, YEp2, YEp4, YIp5, YEp6, YRp7, YEp20, YEp21, YEp24, YIp25, YIp26, YIp27, YIp28, YIp29, YIp30, YIp31, YIp32 and YIp33.

EK2 Plasmid Systems. The *E. coli* K–12 strain chi–1776. The following plasmids are certified for use: pSC101, pMB9, pBR313, pBR322, pDH24, pBR327, pGL101, pHB1. The following *E. coli/S. cerevisiae* hybrid plasmids are certified as EK2 vectors when used in *E. coli* chi–1776 or in the sterile yeast strains, SHY1, SHY2, SHY3 and SHY4: YIp1, YEp2, YEp4, YIp5, YEp6, YRp7, YEp20, YEp21, YEp24, YIp25, YIp26, YIp27, YIp28, YIp29, YIp30, YIp31, YIp32, YIp33.

EK2 Bacteriophage Systems. The following are certified EK2 systems based on bacteriophage lambda:

Vector	*Host*
λgt*WES.* λB′	DP50*supF*
λgt*WES.* λB*	DP50*supF*
λgtZJ*vir.* λB′	*E. coli* K–12
λgtALO. λB	DP50*supF*
Charon 3A	DP50 or DP50*supF*
Charon 4A	DP50 or DP50*supF*
Charon 16A	DP50 or DP50*supF*
Charon 21A	DP50*supF*
Charon 23A	DP50 or DP50*supF*
Charon 24A	DP50 or DP50*supF*

Appendix G—Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Toxins for Vertebrates

1. *General Information.*

Appendix G specifies the containment to be used for the deliberate cloning of genes coding for the biosynthesis of toxins for vertebrates. Cloning of genes coding for toxins for vertebrates that have an LD_{50} of less than 100 nanograms per kilogram body weight (e.g., the botulinum toxins, tetanus toxin, diphtheria toxin, *Shigella dysenteriae* neurotoxin) should not be performed. No specific recommendations other than those given in Part III of the Guidelines shall apply to the cloning of genes if the protein specified by the gene has an LD_{50} of 100 micrograms or more per kilogram of body weight. Experiments involving genes coding for toxins with an LD_{50} of 100 micrograms or less per kilogram body weight should be registered with ORDA prior to initiating the experiments. A list of toxins classified as to LD_{50} is available from ORDA. Testing procedures for determining toxicity of toxins not on the list are available from ORDA. The results of such tests should be forwarded to ORDA, which will consult with the *ad hoc* Working Group on toxins prior to inclusion of the toxin on the list. (See Section IV–E–1–b–(3)–(i).)

2. *Recommended Containment Conditions for Cloning of Toxin Genes in E. coli K–12.*

(a) Cloning of genes coding for toxins for vertebrates that have an LD_{50} in the range of 100 nanograms to 1000 nanograms per kilogram body weight (e.g., abrin, *Clostridium perfringens* epsilon toxin) should proceed under P2 + EK2 or P3 + EK1 containment conditions.

(b) Cloning of genes for the biosynthesis of toxins for vertebrates with an LD_{50} in the range of 1 microgram to 100 micrograms per kilogram body weight should proceed under P1 +EK1 containment conditions (e.g., *Staphylococcus aureus* alpha toxin, *Staphylococcus aureus* beta toxin, ricin, *Pseudomonas aeruginosa* exotoxin A, *Bordatella pertussis* toxin, the lethal factor of *Bacillus anthracis,* the *Pasteurella pestis* murine toxins, the oxygen-labile hemolysins such as streptolysin O, and certain neurotoxins present in snake venoms and other venoms).

(c) Some enterotoxins are substantially more toxic when administered enterally than parenterally. The following enterotoxins should be subject to P1 + EK1 containment conditions: cholera toxin, the heat labile toxins of *E. coli, Klebsiella,* and other related proteins that may be identified by neutralization with an antiserum monospecific for cholera toxin, and the heat stable toxins of *E. coli* and of *Yersinia enterocolitica.*

3. *Containment Conditions for Cloning of Toxin Genes in Organisms Other than E. coli K–12.* Requests involving the cloning of genes coding for toxins for vertebrates in host-vector systems other than *E. coli* K–12 should be submitted to ORDA for evaluation. ORDA will consult with the *ad hoc* working group on toxins. (See Section IV–E–1–b–(3)–(j).)

Appendix H [Deleted]

Annex A.—Original Proposal of Drs. David Baltimore & Allan Campell; Proposal To Convert the NIH Guidelines Into a Non-regulatory Code of Standard Practice and To Reduce the Recommended containment Levels For Some Experiments

Proposals

(1) Section I–A of the NIH Guidelines will be replaced with the following:

"I–A. *Purpose.* The purpose of these Guidelines is to specify standard practices for constructing and handling (i) recombinant DNA molecules and (ii) organisms and viruses containing recombinant DNA molecules. Adherence to these standards by all laboratories using recombinant DNA is recommended."

(2) Part I–C of the NIH Guidelines shall be eliminated.

(3) Part III of the Guidelines will be replaced with the following:

"Part III discuss experiments covered by the Guidelines. The reader must first consult Part I, where listings are given of prohibited and exempt experiments.

"Where there are existing recommended physical containment levels applicable to non-recombinant DNA experiments with either the host or the vector (such as those specified by the CDC Guidelines), recombinant DNA experiments should be carried out at containment levels at least as high as those recommended for non-recombinant DNA experiments. Otherwise, all non-prohibited experiments may be carried out under conditions of Pl physical containment. As a general practice, investigators should use the highest level of biological containment (HV3>HV2>HV1) which is available and appropriate for the purposes of the experiment.

"Specific exceptions to the prohibitions may be approved by the Director NIH (section I–D). The Director will consider

requests for exceptions from individuals, institutions or corporations regardless of whether the applicant is affiliated with or supported by NIH. Such exceptions will generally be approved for specified levels of physical and biological containment."

This will be followed by a listing of those exceptions which are presently authorized and the containment levels approved for the excepted experiments.

(4) Part VI of the NIH Guidelines shall be eliminated, with the following exceptions:

(a) Those definitions listed in Part IV–C which may be needed clarify statements made elsewhere in the Guidelines shall be retained.

(b) Those portions of Part IV–E defining the composition of RAC and prescribing rules for RAC procedures shall be retained.

(c) The following statement shall be added:

"Each institution conducting or sponsoring recombinant DNA research should take responsibility for monitoring its own activities in this area. Any unusual events that might be associated with the use of recombinant DNA molecules should be reported to the Director, NIH."

(5) Section VI of the Guidelines will be eliminated, except for those portions of section VI–F relevant to the protection of proprietary information submitted in support of requests for exceptions from the prohibitions.

Explanation and Justification

The action has two major effects:

(A) It revokes the mandatory nature of the Guidelines by eliminating those sections specifying regulatory procedures and their underlying organizational machinery. These Guidelines would then resemble the CDC Guidelines in setting standards and providing guidance rather than in regulating the performance of experiments. This purpose is accomplished by items, 1, 2, 4 and 5 of the proposal.

Item 5 (elimination of most of section VI) is included because, with the elimination of section IV, compliance with the Guidelines will effectively become voluntary for all individuals, regardless of NIH support. Special provision for voluntary compliance by individuals and institutions not supported by NIH then becomes superfluous.

(B) It reduces the prescribed level of physical containment for most experiments to P1. This purpose is accomplished by item 3.

These two changes are justified from the following considerations:

(A) *Elimination of Regulatory Procedures.* Opinions differ as to the widsom of the actions and arguments which led to the adoption of the NIH Guidelines in 1976. However, there is fairly general agreement on two points: (i) The establishment of Guidelines has had some beneficial effects. In particular, it has raised the general level of awareness among investigators and institutions of the importance of considering possible hazards that might arise during microbiological research. (ii) Since 1976, neither experimental evidence nor solid theoretical arguments have been advanced to support the position that recombinant DNA research poses any danger to human health or to the integrity of the natural environment.

At this point, we doubt that the beneficial side effects of continued regulation justify the expenditure of time and money required to maintain a regulatory apparatus that has been developed to protect society from hazards that appear to be non-existent.

(B) *Reduction of Recommended Containment Levels.* In the absence of known or suspected hazards, it seems unjustified to single out certain classes of experiments as requiring elevated levels of physical containment. The cost, in discouraging variety and innovation and thereby limiting access to useful knowledge, is real, whereas the benefit is likely to be zero. The use of P1 containment, together with the highest available level of biological containment appropriate to the experimental purpose, will keep the probability of escape and establishment very low without interfering with the conduct of most research.

The prohibitions remain in force. Although we consider it unlikely that experiments in the prohibited categories will generate serious hazards, they represent the one area of the Guidelines which is addressed to risks whose nature can be specified, and that are in principle assessable. Restructuring of some of these categories aimed at delineating areas of real concern is desirable and is currently underway in the case of toxin genes. The results of such restructuring would be to define additional exceptions from the prohibitions, which would then appear in Part III of the Guidelines as amended by this measure.

Annex B.—Documents Prepared by Working Group on Revision of the Guidelines

Summary of Committee Actions, Report to RAC

At the April 1981 RAC meeting, a working group was established to consider major revisions of the recombinant DNA Guidelines. The working group, appointed by RAC chairman Ray Thornton, consists of 13 members, 9 of whom were RAC members as of June 1980 (2 have since ended their terms), 2 liaison RAC members, and 2 other scientists who have had long involvement with the recombinant DNA issue. The list of members is attached. The working group is chaired by Susan Gottesman. Two meetings were held: one on June 1, 1981, at which 8 members were present, and one on July 9, 1981, at which 11 members were present. Minutes of both meetings are available. There was a clear consensus by the working group that some major restructuring of the guidelines was appropriate. There was major disagreement about how far such a revision should go. The basic issues are: (1) Are there qualitatively unique dangers associated with Recombinant DNA research? and (2) If so, what response is necessary for guarding against such hazards; is some special procedure required? If there are not qualitatively unique dangers involved, are the remaining risks adequately addressed by already existing procedures for dealing with research dangers? If not, should one use the recombinant DNA issue to develop appropriate procedures for the more general issues?

After an analysis of the risks (detailed in the accompanying document), the following general conclusions were reached:

(1) Accidental combinations of genes, rising out of "shotgun" cloning experiments or experiments where expression is not specifically engineered, are extremely unlikely to lead to serious problems. In most organisms, the barriers to expression of foreign genes, the necessity for new enzyme activities to function as an integrated part of an existing pathway, and the selective disadvantage of carrying recombinant DNA, will interfere with such organisms establishing themselves in the environment and thus ultimately with their potential to cause harm. Therefore, for these experiments, the minimal controls associated with good laboratory practice should be sufficient. Many such experiments have already been exempted by the NIH from any special procedures.

(2) A particular subset of experiments may pose some possibility of risk. In these experiments, the expression of foreign functions may have been deliberately increased, or normal functions will have been engineered to operate more efficiently. While there is no evidence that this risk is qualitatively

different from the risks associated with other kinds of genetic research, the end result may be an increase in virulence, host range, or survivability of some pathogens.

Given these conclusions, the majority of those present at the July 9 meeting supported a proposal which adopts the containment provisions of the Baltimore-Campbell proposal, but retains the mandatory aspect of the guidelines. A minority preferred either a "voluntary code of practice" as stipulated in the original Baltimore-Campbell proposal, or an end to all specifications for working with recombinant DNA, apart from those stipulations of "good laboratory practice."

The text of the proposal as approved by the subcommittee, with a comparison to current (July 1, 1981) guidelines is attached. In addition, an examination of the state of our knowledge about recombinant DNA risks, to be used as a rationale for change, is attached.

We suggest the following procedures; (1) RAC consider and modify if necessary, the working group's proposal and the supporting rationale statement, (2) The modified proposal and rationale be published in the **Federal Register** and elsewhere, if appropriate, for public comment, (3) All comments be considered by the working group and recommendations for change, based on the comments, be made. The final version should once again be published in the **Federal Register**. (4) RAC take final action on the proposal. This might occur at the January 1982 RAC meeting, or possibly at the following meeting, if more time for comment is considered necessary.

Recombinant DNA Advisory Committee Working Group on Revision of the Guidelines, National Institutes of Health

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Executive Secretary

Gartland, William J., Jr., Ph. D., Director, Office of Recombinant DNA Activities, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205, (301) 496–6051

Revision of the July 1, 1981 Guidelines Proposed by Working Group on Revision of the Guidelines, July 9, 1981

(1) Section I–A of the Guidelines would be amended to read as follows:

I–A. *Purpose.* The purpose of these Guidelines to specify standard practices for constructing and handling (i) recombinant DNA molecules and (ii) organisms and viruses containing recombinant DNA molecules.

(2) Section I–D of the Guidelines, *Prohibitions,* would be eliminated.

(3) Part III of the Guidelines would be replaced with the following language:

Part III discusses experiments covered by the Guidelines. The reader should first consult Part I, where exempt experiments are listed.

Where recommended physical containment levels applicable to non-recombinant DNA experiments exist for either the host or the vector (such as those specified by the CDC Guidelines, or the USDA Quarantine Regulations), recombinant DNA experiments should be carried out at containment levels at least as high as those recommended for non-recombinant DNA experiments. If there is clear evidence that the donor DNA will significantly change the pathogenicity of the host, the containment level appropriate to the anticipated change will be applied. Otherwise, all experiments may be carried out under conditions of P1 or P1–LS physical containment.

(4) Those membership specifications of the IBC to be found in Section IV–D of Section IV of the Guidelines would be eliminated.

BILLING CODE 4110-08-M

OUTLINE OF CURRENT NIH GUIDELINES FOR RECOMBINANT DNA RESEARCH AND PROPOSED CHANGES

	Current Guidelines: Procedures	Subcommittee Proposal	Baltimore-Campbell Proposal
I. Prohibitions (I-D)			
CDC Class 4 & 5 organisms Genes for potent toxins Release to environment Genes for drug resistance	Prohibitions take precedence over all other parts of guidelines; exceptions possible by application to RAC and Federal Register publication	Eliminate prohibitions as special class; handled by IBC as with other experiments	Retains prohibitions
10 liters unless well characterized and harmless	This prohibition does not take precedence over exemptions 1-4		
II. Exemptions (I-E)			
Naked DNA Rearrangements Self-cloning Exchangers Others: Tissue culture, *E. coli*, *B. subtilis* and *S. cerevisiae* HV1 systems	No special oversight required for these experiments; new additions to list by application to RAC and publication in Federal Register	No change	No change
III. HV1 Systems (III-A)			
Currently covers only some *N. crassa* and *Streptomyces* strains	Containment varies with source of donor DNA (P1-P3). New systems require approval by RAC and Federal Register publication	P1	P1
IV. All other prokaryote, lower eukaryote combinations	P3; lower by request to ORDA. Return to host of origin, P1	P1 for nonpathogens; pathogens as per CDC, etc.	P1 for nonpathogens; etc.
V. Eukaryote recipients			
non-viral vectors	P1-P2 in whole organ. (exempt in tissue culture)	P1 for non-pathogens, etc.	P1 for non-pathogens, etc.
defective viral vectors	P1-helper, P2-P3 + helper		
non-defective viral vectors	case-by-case; (P2 in most cases)	P1 for non-pathogens, etc.	P1 for non-pathogens, etc.
Procedural Changes: IBC	membership specified	no specifications for membership	voluntary system

BILLING CODE 4110-08-C

Evaluation of the Risks Associated With Recombinant DNA Research

I. History and Introduction

The technique known as recombinant DNA or gene cloning was first developed in the early 1970s. It provides methods for combining pieces of DNA from essentially any source—no matter how unrelated—and reintroducing them into living cells. In practice, in order to ensure that the newly joined DNA persists in its host, small pieces of the DNA of interest are joined to a DNA (a vector) which has the capacity for self-replication in the host cell.

The development of this incredibly powerful new technique led thoughtful scientists, aware of the history of use and abuse of similar breakthroughs in the fields of chemistry and physics, to raise to their fellow scientists questions about the possible hazards of the technique. This occurred before the technique had been used enough to allow those talking about the dangers to have any expectations about what the full possibilities or complications of the experiments might be. The initial discussion raised enough concerns for scientists to ask, first, for a moratorium on some sorts of experiments, and secondly, for the development of guidelines for the use of recombinant DNA technology. The form of the initial restrictions was outlined at the meeting of 150 scientists held at Asilomar in February 1975. This document served as informal guidance to scientists working in the field until the issuance of the first NIH Recombinant DNA Guidelines in June of 1976 (**Federal Register,** July 7, 1976). The impetus to obey these Guidelines was both peer pressure and the threat of withdrawl of NIH grant funds

The original Guidelines relied on the use of physical and genetic containment procedures as safeguards for dealing with unspecified dangers. As the possibilities of the recombinant DNA technique were more fully realized, it became clear that many experiments were not mentioned in the original guidelines either because they had not been of particular concern to those who wrote the Guidelines, or because the necessary experimental techniques had not yet been developed. On the other hand, large classes of experiments, particularly those with animal viruses, had been extremely restricted. Because of a concomitant rise in public awareness and concern about the whole of recombinant DNA experimentation, NIH at this stage felt it appropriate to approve only experiments explicitly discussed in the 1976 NIH Guidelines. Scientists were frustrated that experiments they both knew how to do and knew would lead to rapid advances in subjects of major importance were blocked. In addition, they felt under attack as public scrutiny of the molecular biology field sometimes implied that no scientist could be trusted to supervise his own work and that yet stronger control would be necessary to properly guard the world against the dangers of recombinant DNA. As the original concept of care and self-policing were replaced by complex regulations and restrictions scientists perceived as inappropriate, support for the guidelines among scientists decreased dramatically.

Simultaneously, the NIH was developing the first major revision of the Guidelines to deal with inconsistencies and changing perceptions. The first major revision took more than two years from start to adoption in late 1978 (**Federal Register**, December 22, 1978). Major changes were introduced. They included: Creation of an "exempt" category for experiments considered to be innocuous, the lowering of containment requirements for experiments with animal viruses, and the delegation of more oversight authority for recombinant DNA experiments to local committees (IBCs). In addition, the new Guidelines explicitly recognized the need for a constant evolution of the Guidelines and specified a procedure for such change. That gradual evolution has continued over the last three years, and has included some fairly radical changes in the range of permitted experiments, the level of containment requirements for experiments, and the procedures for administration of the Guidelines. The trend has been to remove from most requirements, those classes of experiments for which no specific danger can be hypothesized.

At present, five years after the first Guidelines went into effect and seven years after the Asilomar Conference, the situation is as follows (**Federal Register,** July 1, 1981):

(1) The Guidelines permit a large number of experiments using the recombinant DNA technique, and interfere with and slow ongoing investigations in most areas far less than they did in 1976–1978;

(2) Many experiments, especially those in *E. coli* K–12, *Saccharomyces cerevisiae,* and *B. subtilis* host-vector systems, can be carried out at low containment, and with relatively few registration or prior approval procedures;

(3) Some recombinant DNA experiments, especially in organisms which are not as well studied as the originally used *E. coli* K–12 host, still require special permission from and discussion with an oversight committee, either at the local or national level;

(4) A great deal more experience with the limits and possibilities of the recombinant DNA technique, seven years of explicit discussion of the possibilities for harm and how these possibilities can be evaluated, as well as some NIH supported risk assessment studies have provided scientists somewhat better parameters for discussing the dangers of recombinant DNA;

(5) Experience with regulation of the recombinant DNA field over the last five years should provide some information about the effectivness of various provisions if such regulation is needed;

(6) The Guidelines have evolved into an extremely complicated, piece-meal document in response to modifications initiated by specific, single-issue requests. Most scientists experience difficulty in picking their way through this document.

(7) Administration and revision of the Guidelines continues to require scientists to expend considerable time, both as part of the RAC and the IBCs, and in the laboratory.

In response to a specific proposal by two members of the NIH Recombinant DNA Advisory Committee, that "the time for Guidelines with attached oversight procedures and penalties has passed," the RAC has designated a study group to evaluate what we now know about the possible dangers of recombinant DNA, how those dangers are met by the Guidelines as they now stand, and whether such Guidelines are still appropriate for this scientific technique. Below, we will analyze the basic assumptions and types of risk which were visualized, and what has been learned to either allay or reinforce these fears. We will then analyze the possibilities for dealing with remaining concerns, ranging from continuation of the current Guidelines through abolition of any special oversight.

II. Possible Hazards

A. Basic Assumptions

In some ways, recombinant DNA poses a unique problem. One can add new genetic information to practically any organism, and arrange matters so that new information is stably maintained and possibly expressed in the host. In addition, because we are dealing with viable, self-replicating

organisms, the altered individual would not necessarily sit on the inventor's shelf, like some new and terribly explosive chemical, but might be able to establish itself in the environment and, because of its ability to replicate, magnify any harmful effect. Three assumptions are therefore involved in the decision to call recombinant DNA a unique danger; each one of these assumptions can be separately discussed and evaluated. These assumptions are:

(1) That a unique organism, never found in nature, might be constructed by recombinant DNA techniques;

(2) That such a unique organism might be able to establish itself in the environment outside the laboratory;

(3) That such an organism, established in the environment and possessing unique properties bestowed upon it by recombinant DNA techniques, might be harmful, either to man, animals, or plants.

If one could prove that any one of these three assumptions is totally false, the dire expectations of the recombinant DNA technique could be discarded and no special precautions would need to be taken. In the initial assessment, however, these assumptions seemed reasonable, in that the technique appeared to allow any organism to be mofidied in any way.

Over the last seven years, some changes in perception and in knowledge of recombinant DNA hazards have curred:

(1) The possibilities and limits of assumption 1 are somewhat better understood in (a) the limits to which recombinant DNA can bestow new characteristics on organisms, and (b) the ways by which organisms normally exchange genetic information in anticipation of the recombinant DNA technique, thereby negating the "uniqueness" component of the assumption.

(2) A great deal of discussion in the last few years has centered on assumption 2; the whole concept of biological containment is based upon circumventing the organisms' establishment in the environment by modifying the properties of the organism. In the case of *E. coli* K–12, specific tests to asses the likelihood of establishment, with or without recombinant DNA, have been carried out (see appendix), and have suggested that such establishment is unlikely. In the more general sense, can any change made in the laboratory either intentionally or unintentionally endow an organism with a competitive advantage in a very competitive outside environment? Seven years experience with recombinant DNA has suggested that recombinant DNA is frequently not stably maintained in the host organism in the absence of selective pressures.

(3) Assumption 3, that such a unique established organism will cause harm, remains primarily one of discussion, since experiments where one might explicitly predict harm have been for the most part forbidden or discouraged. Some explicit experiments to assess risks have been performed: I.e., examination of whether *E. coli* can act as a vector to introduce viral DNA into animal cells (Israel et al., 1979, a, b; Chan et al., 1979), and whether *E. coli* manufacturing active hormones can affect the physiology of animals (Stebbing et al., 1980). Much of the other discussion involves unknowns, although understanding of the basis for pathogenicity of some organisms can help in predicting the probable effects of introducing new genetic information.

We will try to analyze (1) the kinds of information available to support or disprove each of these assumptions, (2) how these assumptions have been dealt with in past Guidelines, and (3) how they might be dealt with in the future.

The early Guidelines presumed that, since the possibility of harm could not be properly evaluated, all recombinants were potentially harmful; the Guidelines controlled the establishment of recombinant DNA containing organisms in the environment by indicating levels of physical and biological containment. More recently, the Guidelines have evolved to include the concept that only unique organisms should be of concern. This concept is the basis for the long list of exemptions in the 1978 Guideline revision. Other relaxations in the Guidelines concerning *E. coli* K–12 host-vector systems have resulted from further examination of the second assumption, as evidence accumulated on the inability of *E. coli* K–12 to establish itself in the environment.

Accidental vs. Intentional Cloning of a Harmful Segment. One other aspect of the change from early to more recent attitudes can be traced to the qualitative difference between accidental constructs of unknown recombinants and the intentional cloning of a specific gene. The early Guidelines in their concern for inadvertant combinations of potentially harmful genes, required high containment for experiments in which random pieces of DNA from one organism are inserted into a second organism (shotgun experiment). Advances in our understanding of how expression of mammalian genes (eukaryotes) differs from expression of genes in lower forms of life such as bacteria (prokaryotes), has led to a perception that the accidental altering of an organism is very unlikely to produce a harmful result. Thus, some lessening of containment for such "shotgun" experiments has seemed justified. The other concern, and probably the basis for the remaining concerns, centers on the deliberate construction of a recombinant organism expressing a foreign product. The rapidly developing techniques of recombinant DNA technology can be utilized to insure that the recombinant DNA in fact efficiently expresses a product in its new host. The organism can, in addition, be engineered to excrete the product into the outside environment. In such a case, questions about possible harm can be posed without considering assumptions on the likelihood of expression or the possibility of one DNA fragment out of many establishing itself.

1. *Uniqueness of Organisms Created by Recombinant DNA Techniques.*

A. *Limits of the techniques.*

The original assumption that DNA from essentially any source could be introduced into essentially any organism has been supported by research advances of the last six years. Methods have been developed for introducing foreign DNA stably into a variety of bacteria, yeasts, plants and animal cells. In some organisms, the nature of the vector or the growth pressures on the organism limit the amount of new information which can be introduced as recombinant DNA. For example, many viral vectors have size limits on the amount of introduced DNA; only a specific amount can be packaged into viral the coat (Blattner et al., 1977). Some bacterial plasmids become harder to maintain stably in cells as their size increases. In practice, many recombinant DNA experiments are more productive when relatively small, defined pieces of the foreign DNA of interest are used. In considering the general limits for the purposes of risk considerations, it seems fair to say that there is no limit to either the source of DNA (donor) or of the host (recipient) but that size of the donor DNA may be limited, especially where viral vectors are used.

b. *Natural exchange mechanisms.*

The earliest discussions of the recombinant DNA technique considered the well known ability of *E. coli* to exchange DNA with other organisms as a possible hazard of using the recombinant DNA technique in that host-vector system. Such exchange might disseminate recombinant DNA from an *E. coli* K–12 host to other less well characterized and less disabled hosts; therefore, use of plasmids that

could not transfer readily to other hosts (nonconjugative plasmids) were specified by the Guidelines. The 1976 Guidelines did not specifically acknowledge the additional conclusion that such exchange implies: that for at least some pairs of organisms, recombinant DNA experiments may simply imitate nature in moving blocks of genes from one species to another.

A series of promiscuous plasmids that move freely among severl species of bacteria, have been found in a large series of gram negative organisms, ranging from *E. coli* through *Rhizobium* and *Pseudomonas* (Alexander & Jollick, 1977; Haas & Holloway, 1978; Baron et al., 1968). These plasmids frequently carry, in addition to genes for their own maintenance and transmission, antibiotic resistance genes and occasionally chromosomally derived genes (Olsen & Gonzalez, 1974; Holloway, 1978). Evidence of *in vivo* transfer of these plasmids from virtually any gram-negative species to any other gram-negative species has been found (Ingram et al., 1974; Smith, 1969). In laboratory experiments, chromosomal genes from a variety of organisms can be transferred by these plasmids into other species (chromosome mobilization). In some cases, such chromosomal genes can be stably recombined into the recipient chromosome. In other cases, the mobilized genes can be found stably associated with the plasmid, and, therefore, can be transferred at high efficiency to many organisms (Holloway, 1978).

Although the precise nature of this mobilization is not know, it seems to be somewhat plasmid specific. At least some plasmids can mobilize the DNA of many diverse organisms (Haas & Holloway, 1978), and many genes from any given organism can be mobilized by these plasmids. Given the ubiquity of such plasmids, the ample opportunity for exchange in the environment these organisms share, and the very long time periods available, it seems reasonable to assume that, for most gram-negative bacteria, any given gene has in fact been introduced at some time into any given recipient. This probability was explicitly recognized in the 1978 revision of the Guidelines by the inclusion of a list of "exchangers" (Federal Register, July 28, 1978). Recombinant DNA experiments between two exchangers are exempt from the Guidelines on the rationale that such a pair should have been exposed to each others DNA, and, therefore, no unique combination should be produced via recombinant DNA technology. If this principle is accepted in the broadest sense, essentially any cloning among the enterobacteriaceae, both pathogenic and non-pathogenic, would be exempted from the Guidelines.

Many gram positive bacteria take up DNA from the environment (Low & Porter, 1978). When the DNA taken up has similar sequences to the host bacteria DNA, the bacteria are capable of recombining that DNA into their own chromosome. Some of these organisms have been shown to be transformed (changed genetically) by DNA released by neighboring bacteria in the soil (Graham & Istock, 1978; Burke & Le, 1980). Therefore, such organisms exchange genetic information with other organisms by transformation; some pairs of microorganisms have been added to the exchanger list on this basis.

Similarly, bacteria may frequently encounter mammalian DNA, either from decaying matter or from intestinal cells or ingested food in the case of gut bacteria. Mammalian cells in the intestine should frequently be exposed to DNA released from resident bacteria. It is difficult in these cases to realistically estimate uptake and persistence, although mechanisms for "illegitimate recombination" do exist both in bacteria and mammalian cells (Cold Spring Harbor Symposium, 1981; Kleckner, 1981). Large pieces of non-homologous DNA can be incorporated into the mammalian genome (Scangos & Ruddle, 1981).

There have been reports of the existence of mammalian hormone-like proteins elaborated by bacteria (Koide & Maruo, 1981; LeRoith et al., 1981); this may reflect uptake and integration of mammalian DNA by bacteria sometime in the past.

In at least one case, a system for genetic transfer from bacteria to plants exists. The plant pathogen *Agrobacterium tumefaciens* transfers a specific part of its extrachromosomal DNA, implicated in plant tumor formation, stably to the infected plant cells where the DNA is expressed and stably maintained (Chilton et al., 1977). The transferred piece is derived from a large, transferable plasmid which has the capacity to enter, by conjugation, many other prokaryotes. It is not known whether other such mechanisms exist, but even this isolated example provides a mechanism for moving bacterial and perhaps other types of information into many kinds of plants.

Among animals and plants, viruses seem to be the most likely mechanism for pick up of chromosomal genes and transfer of genetic information from one host to another. In the last few years, it has become clear that many chromosomal sequences can be found in association with viral DNA (Bishop, 1981; Weinberg, 1980).

c. *Counterarguments.*

i. While exchange can be documented in the laboratory, most genes will not be transferred *in vivo* in stable association with plasmids; only the most homologous regions will recombine into the recipient chromosome. Species that do not occupy the same ecological niche are unlikely to have the opportunity for natural exchange of genetic information. Introduction of DNA via transformation will rarely lead to stable diploid formation or replacement of the original DNA with new sequences. The use of the recombinant DNA technique may increase these processes dramatically by providing a mechanism for maintaining new sequences in the absence of homology.

ii. The vectors used for recombinant DNA experiments may be engineered to exist in many copies in the cell, or other mechanisms may be used to increase expression of genes beyond that found in nature. Such optimization of expression could lead to an organism unique in its ability to produce an excess of a particular product.

2. *Dissemination.*

a. *Stability of Recombinant DNA.* Much of the discussion of the possible hazards associated with recombinant DNA in *E. coli* K-12 has centered on the inability of this organism to establish itself in the environment or disseminate recombinant DNA to other organisms. We have summarized some of these data in the Appendix, and further discussion of the data can be found in the report of a meeting in Falmouth, Massachusetts, where the epidemiological consequences of cloning in *E. coli* K-12 were discussed (Gorbach, 1978).

Some of the arguments made with respect to *E. coli* K-12 can be generalized to other systems as well. Laboratory strains, which most frequently will be used as hosts for recombinant DNA, may lose some of the characteristics which permit growth outside the laboratory. Many vectors, in being redesigned as useful vectors for recombinant DNA experiments, will lose the capacity for self-transmission. However, in a discussion of whether essentially any recombinant DNA containing organism is likely to be disseminated, we must assume that at least in some cases the host will be one not far removed from the wild environment, and that, in some cases, self-transmissible vectors will be used to carry the recombinant DNA. Therefore, one must ask if there are

general arguments about the ability of the organism carrying recombinant DNA to compete successfully with nonrecombinant DNA organisms.

There is a great deal of anecdotal information about the difficulties that people have maintaining recombinant DNA in bacterial strains; selective pressures must be applied continuously to maintain many plasmids. Little of this type of information has been published. One such experiment, reported by Cameron and Davis (1977), examines the fate of random *E. coli* and *Saccharomyces cerevisiae* fragments cloned into a bacterial virus vector and propagated for many cycles in *E. coli*. After about 25 cycles of growth, both sets of DNAs were reduced from the original diverse population to one or two dominant types which presumably have a growth advantage over all the other types. The authors also state that none of the yeast clones outgrow the parent vector (containing no recombinant DNA information). Therefore, most recombinants grown under these conditions will be quickly lost after the host organism begins to multiply.

Evolutionary arguments suggest that an organism containing recombinant DNA information will be at a relative disadvantage; this is particularly true for complex organisms such as mammals. Ayala (1977) points out that, for developmentally advanced organisms, new information must be coadapted to the rest of the gene pool of that organism; this is almost impossible to do with new information unless the new information is simply a different form of an already present gene. If, however, the new information is very similar to that which is already present, such a variant might have arisen by natural means and the recombinant DNA containing organisms will not be unique. Chances for altering the evolution of simpler organisms, such as prokaryotes, may be somewhat greater, although short generation times and relatively economical use of DNA sequence information suggests that non-useful information will be rapidly lost. Overproduction of one or a few products would be expected to unbalance the cell's metabolism.

b. *Transmission into other potential hosts. There are three major mechanisms for transfer of recombinant DNA from the original host to other hosts encountered in the environment.*

(1) For plasmids, in particular, the conjugational mode (by mating, involving cell-to-cell contact) may be primary. Either conjugative transfer of a self-transferring plasmid or mobilization of non-conjugative plasmids may occur (Low & Porter, 1978). At least for one class of vectors frequently used for recombinant DNA, those based on the *E. coli* plasmid pBR322, it has been demonstrated *in vitro* and *in vivo* that transfer by mobilization is greatly reduced (Dougan, G., Crosa, J.H., Falkow S., 1978; Levine, M.M., Kaper, J.B., et al., unpublished data) apparently because a segment necessary for mobilization was deleted in construction of the vector (Clark & Warren, 1979). Clearly the range of possible recipients will depend on the host range of the transferring plasmids.

(2) Viral vectors, if they are intact, may readily transfer the recombinant DNA they carry to other sensitive hosts. If the host is killed in the process, the association will be only a temporary one. If the vector is defective (lacking an essential function), the function must be supplied by a helper virus for each new round of growth and infection. Therefore, if an appropriate helper is not commonly found in the environment, it is unlikely that the defective viral vector and its recombinant DNA will be disseminated.

(3) Finally, cells are capable of taking up naked foreign DNA. For bacterial systems carrying out transformation, restriction systems will frequently degrade the incoming DNA if it is from a foreign strain, thereby reducing greatly the probabilities for stable incorporation of incoming recombinant DNA. In addition, it has been found that plasmids serve as a particularly poor source of DNA for transformation into some strains (*B. subtilis*) (Canosi et al., 1978; Contente & Dubnau, 1979). Since DNA which is nonhomologous and is not competent to replicate by itself will not be recombined into the host chromosome or maintained in the cytoplasm, it may be reasonable to assume that naked recombinant DNA taken up by prokaryotes by transformation will not generate stable recombinant DNA containing organisms. In addition, at least in some environments, nucleases, pH and other environmental factors should interfere with the stability of naked recombinant DNA. In one experiment, bacterial DNA exposed to the diluted contents of rat intestine was rapidly degraded (Maturin and Curtiss, 1977). In other cases, however, *in vivo* DNA transformation has been observed, suggesting that not all DNA released into the animal tissues will be immediately destroyed (Ottolenghi-Nightingale, 1969).

Take-up of DNA by animal cells in tissue culture is not an efficient process under optimized laboratory conditions (Scangos & Ruddle, 1981). Moreover, for an effective cycle which will lead to wide-scale dissemination, the DNA would have to be stably integrated into the germ line of an intact organism. Alternatively, some constant source pool, such as infecting bacteria or viral recombinants may continue to provide a source of the DNA.

Individual plant cells, since they can be regenerated into complete plants, could disseminate DNA if it were integrated into nuclear or organelle DNA. However, this may also require a constant bacterial or viral source pool in plant cells.

3. *Harm.*

Let us assume for the moment that the organisms we have created via recombinant DNA are in fact unique, and have had the opportunity and ability to establish themselves in the environment. Will these organisms have the ability to cause harm, either to ourselves, to other animals, or to plants, microorganisms and the environment? We will consider here some general arguments about the roles recombinant DNA can play, and some of the specific cases which people have considered to be areas of concern.

a. *Breaching prokaryotic-eukaryotic barriers: evolutionary considerations.*

Prominent among the concerns expressed during the first few years of recombinant DNA technology was the fear of "breaching the barriers" to permit recombination between distantly related organisms. This concern, articulated most prominently by Sinsheimer (1975, 1976 a, b) and by Chargaff (1976), implies that the fertility barriers that evolved as organisms diverged during evolution arose to prevent the creation of new, dangerous species. Davis argues that this view "turns evolutionary principles upside down. Evolution has indeed established fertility barriers between species. But these barriers do not function to prevent the formation of *monsters* that might take over in the Darwinian struggle: They prevent wasteful matings that would produce only unbalanced *monstrosities*, unable to survive" (Davis, 1976, 1977).

In addition, information based primarily on the recombinant DNA analysis of eukaryotic genomes makes it clear that the mechanisms for processing information from DNA into protein differ greatly between many eukaryotes and prokaryotes (Breathnach & Chambon, 1981; Revel & Groner, 1978). Therefore, it is difficult to imagine that for most cases the "breaching of the brarrier" will result in much more than adding silent DNA sequences into the host cell. In the absence of any expressed function, it is difficult to hypothesize either a beneficial or

harmful effect of eukaryotic DNA for a prokaryote. Therefore, experiments which do not include the intentional expression of eukaryotic DNA in prokaryotes, or prokaryotic information in eukaryotes, should be considered unlikely to express a product; any harm would have to be due to the DNA itself, and any competitive advantage for survival would likewise have to be due to the DNA itself. We will consider below those cases where the recombinant is designed to express a foreign product.

b. *Small pieces in large organisms.*

Many recombinant DNA experiments involve the introduction of pieces of relatively small foreign DNA into a host. Thus, for many experiments in *E. coli,* pieces which represent no more than 0.5–1% of the recipient's genetic information are added as recombinant DNA. In most cases, this kind of new information, unless carefully integrated with information already present, will not significantly alter the ecology of the organism (Ayala, 1977). Carried further, this argument may suggest that new information introduced into a pathogen is not likely to significantly change its mechanism of pathogenicity. Information which must be processed to serve as part of a more complex biochemical pathway, as is often the case for the surface components which play an important role in bacterial pathogenesis, will be even more difficult to alter via recombinant DNA.

For more complex cells, the added DNA will represent a small part of the recipient's genome. This argument clearly does not hold as well for viral recombinants, where the added recombinant DNA information may be equal in quantity to what is already present. In addition, it may not apply if one is speaking of planned changes, such as introducing antibiotic resistance genes or causing overproduction or change in some normal cell component; i.e., increasing toxin synthesis, or altering the characteristics of a surface component of a bacterial cell such that the antigenicity of the cell will be changed.

c. *Specific Cases.*

i. *Expression of Active Peptides: Hormones, Toxins.* One major class of concerns in the discussion of the risks of recombinant DNA is based on the assumption that bacteria could be programmed to express, in large quantities, active proteins which would cause, either by themselves or through their antigenicity, some untoward reaction in their host. This issue was specifically addressed for *E. coli* hosts at a meeting sponsored by NIAID in Pasadena, California in April 1980. Since for the sake of discussion the participants assumed the "worst case" of transfer of the recombinant DNA to an established colonizing organism, some of the conclusions from that meeting can be generalized to the case of cloning in the prokaryotes which colonize the intestinal tract. Reports from that meeting have been published in the *Recombinant DNA Technical Bulletin* (Volumes 3 and 4, 1980–1981) and are summarized here.

If one assumes transfer and maintenance of a recombinant plasmid in essentially all *E. coli* of the intestinal tract one can calculate that the bacteria, at their maximum synthetic capacity, will produce in the range of 10^6 molecules/cell/generation, or about 50 micrograms a day of a product of the size of insulin. Given this number as an upper limit, one can calculate for various active peptides the maximum dosage the hose will receive and the effect on the host from these dosages. Such calculations suggest that most hormones with activities similar to insulin will not be expected to have much, if any, effect on a mammalian host, even if they are exported from the bacterial factory and absorbed from the intestine in active form. Much more active peptides or proteins, however, might be of some concern under such circumstances. If, in addition, one imagined as the host anaerobic bacteria as well as or instead of aerobes, the body dose of an expressed protein might be significantly greater.

Fifty micrograms per day, on the other hand, of the most active toxins (e.g., the botulinum toxins, *Shigella dysenteriae* neurotoxin, tetanus toxin, diphtheria toxin) is well above the intravenous lethal dose for an average man. Several other toxins, including three of plant origin (abrin, ricin and modeccin), may be lethal to man within the range of 10 to 100 micrograms. The effects of the cytotoxic toxins, intraintestinally produced, on the lining of the lower GI tract are generally unknown. They presumably might (1) damage the colonic or intestinal lining directly, (2) pass through the lumen and cause damage elsewhere, or (3) pass into the bloodstream subsequent to lumen damage. Little is known of the pharmacokinetics of toxins in the body.

Thus, these arguments suggest that for cloning of foreign proteins, only the most active kinds of peptides, at maximal levels of expression, would be likely to have some effect, if one ignores the problems of dissemination and establishment.

ii. *Expression of Cross-Reacting Antibodies.* The implication of inserting eukaryotic genetic material coding for human "self" antigens into prokaryotic microbe vectors that parasitize humans was also specifically addressed by the NIAID meeting in Pasadena, California (Workshop on Recombinant DNA Risk Assessment). When considered within the framework of microbial parasitism in its broadest perspective and against contemporary concepts of immunologic tolerance to "self" constituents and host autoreactive immune responses, the injurious potential of autoreactive immune responses elicited by "cross-reacting" infecting host-vector microbes would appear to be extraordinarily low.

This conclusion is supported by the following observations:

- Literally hundreds of cross-reactivities exist between human proteins and bacterial and viral surface proteins. Most of these cross-reacting proteins have not been implicated in autoimmune injury.
- Abundant data exists indicating that some cross-reacting systems do lead to production of autoantibodies. Most of these autoantibodies are low affinity binding antibodies which do not lead to tissue injury and clinically manifest disease. Whether low affinity or high affinity antibodies will be induced by cross-reacting systems displaying human antigens is unknown.
- Some type of regulatory restraint exists which allows "self" to be recognized by host immunocompetent cells but prohibits such cells from launching an "anti-self" immunologic attack (Paterson, 1981).

iii. *Animal Virus Cloning.*

Recombinant DNA experiments involving the use of animal virus genomes fall into several possible categories:

(1) Cloning of entire or partial genomes in prokaryotes,

(2) Cloning of animal virus genomes in eukaryotic cells under conditions where the genome may replicate either autonomously or in concert with the host genome, but cannot produce an infectious particle (defective virus or non-permissive infection),

(3) Cloning of an animal virus genome in eukaryotic cells under conditions where the genome may not only be replicated but also may be matured into an infectious virus.

In all these cases, the animal virus may be considered as either "recipient" or "donor"; the difference is frequently semantic only. General considerations will be reviewed here. A more technical analysis of the issues to each class of animal virus are considered in the report of the Ascot Meeting (**Federal Register,** July 28, 1978).

Cloning of animal viruses is considered here with respect to the question of whether recombinant DNA experiments *per se* pose any unique hazards not associated with handling animal viruses in general. The following comments are offered in light of the consideration that all work with animal viruses poses some potential risk, unrelated to recombinant DNA, especially to investigators. It is essential to employ good laboratory techniques appropriate to the particular virus being studied.

- *Cloning of viral genomes in prokaryotes*

Our increased understanding of the differences in gene expression between prokaryotes and eukaryotes renders it difficult to imagine ways in which complete animal viral particles could be synthesized in recombinant DNA containing bacteria. Animal viruses have complex post-transcriptional modification requirements, and use host functions in processing and assembly which are not provided by a bacterial host.

- *Cloning of Viral Genomes in Eukaryotic Cells*

Because animal cells in culture are so fragile, they in themselves constitute an excellent containment system. The major potential risk would be that a recombinant DNA molecule could replicate, be encapsidated as a normal virus, and be able to propagate as a virus. There are several potential constraints on such a system, primarily the fact that there is an upper limit on the size of the genome which may be encapsidated. Under such conditions, some of the original viral genomes would most probably be deleted, rendering the recombinant virus defective. However, such a defective virus might be propogated in the presence of a helper virus. Such systems are known to exist both naturally (avian retroviruses and adeno-associated viruses) and in the laboratory (adeno-SV40 hybrid viruses, most of which are defective, but may be propogated). Recombinants might include genes from different viruses or cellular genes inserted into a viral genome. Examples of both natural types of recombinants are generated by recombination in cell culture (e.g. the adeno-SV40 hybrid viruses and the highly oncogenic types of both avian and murine retroviruses—those which have incorporated a Src gene of cellular origin). Interestingly, none of the known examples have proven particularly hazardous for experimenters to handle. Presumably a human retrovirus might be especially hazardous but this situation could obtain independently of any recombinant DNA experiments.

Recombinants between viruses which are not closely related (different Families) are likely to have such basic differences in mechanisms of replication and infection as to be unlikely to work together well enough to produce viable entities; therefore the recombinant in this case will be equivalent to, or more defective, than the parent viruses. Recombinants between viruses which are more closely related may be more likely to create new useable functions, but the close relationship may suggest that such a recombination will have already occurred naturally.

In summary recombinant DNA experiments involving animal viruses in eukaryotic cells might well lead to the construction of some viruses with properties which are novel in detail. However, there is no reason to think that these agents would be novel in terms of host range or potential virulence compared to viruses generated in other ways.

Much of the summary of animal virus recombinant DNA risks is based upon the conclusions of virologists at the EMBO meeting in Ascot, England, January 27–28 1978 (Federal Register, July 28, 1978), and at the ASM meeting in Miami Beach, Florida, April 13, 1981. Reports on these meetings are available.

B. Human Genetic Engineering

The implications of the recombinant DNA technique for inserting recombinant DNA into humans has served as a backdrop for much of the recombinant DNA debate. The guidelines as now written deal with this issue only obliquely, by requiring special RAC consideration for most such experiments. Currently, human experimentation falls under the control of human experimentation review groups (Institutional Review Boards, IRBs). If a decision to remove the recombinant DNA oversight of this technique is made, it will still be oversighted by IRBs. It is not clear that recombinant DNA *per se* poses a special problem in this area, distinct from those posed by non-recombinant transformation and genetic manipulation procedures.

III. *Costs*

Expenditures for risk assessment testing, administration of RAC, and the functioning of the Office of Recombinant DNA Activities were approximately $700,000 in the peak year of 1979. Risk assessment costs accounted for approximately one-half of that total. In all, risk assessment and vector development contracts supported by NIAID have cost over $2 million since 1976. There are also expenses associated with the functioning of Institutional Biosafety Committees, and with the oversight of physical containment facilities.

Costs in time and energy for individual scientists are difficult to estimate but continue to be significant.

IV. *Conclusions*

A. Summary Analysis of Risks

Given the above analysis of the risks associated with recombinant DNA, we have come to the following major conclusions:

(1) That accidental combinations of genes, rising out of "shotgun" cloning experiments or experiments where expression is not specifically engineered, are extremely unlikely to lead to serious problems. Both the barriers to expression of foreign genes in most organisms, the necessity for new activities to function as an integrated part of an existing pathway, and the selective disadvantage given to an organism by recombinant DNA inserts will interfere with such organisms establishing themselves in the environment and thus, ultimately with their potential to cause harm. Therefore, for these experiments, the minimal controls associated with good laboratory practice should be sufficient.

(2) A particular subset of experiments may still pose some possibility of risk. While there is no evidence that this risk is qualitatively different from the risks associated with other kinds of genetic research, the possibility for improving the virulence, host range, or survivability of some pathogens does seem to exist. In most cases, in these experiments the problems of expression of foreign functions will have been bypassed, or normal functions will have been engineered to operate more efficiently.

In many cases, even the best engineered strain will be at a major disadvantage in the environment, or will require artificial selections to maintain recombinant DNA information. The issue to be faced here, however, is (a) how serious is this risk? (b) What is the most effective, non-obtrusive mechanism for guarding against any untoward consequences of such work?

B. Possible Responses

Re-evaluation of the guidelines might in theory lead to changes in the guidelines' containment requirements, or the oversight procedures, or both. We will consider here the two extreme cases of (1) maintaining the current

guidelines, or (2) totally abolishing the guidelines. The route recommended by the subcommittee will then be discussed.

1. *Maintain the Status Quo.*

a. *Advantages.* The *status quo* is not static, since the 1978 major revision of the guidelines recognized the need for constant evolution of the guidelines and provided procedures for changes which have been applied frequently in the last three years. The guidelines have been useful; their existence raised the awareness of investigators and institutions as to the importance of considering and avoiding hazards in the laboratory. If there are significant dangers associated with recombinant DNA research, the safety of this research thus for may have been due to the existence of the guidelines. Time will permit accumulation of more experience and data relevant to risk assessment so that reductions in restrictions can be derived from a greater factual base. A carefully thought-through simplification of the guidelines could be one immediate aim, while preserving the current guideline structure.

Gradual evolution of the guidelines would permit exploration of the means of dealing with possible laboratory-created biohazards so that tracking systems for such hazards in general—inclusive, but not limited to, those that might arise from recombinant DNA technology—might be established. To monitor biohazards, an expanded role for a body similar in concept to RAC might also be explored, as well as that body's relation to the IBCs. Current guidelines for good laboratory practices could be examined and possibly adapted to include recombinant DNA experiments.

b. *Disadvantages.* The current guidelines are long, cumbersome, and detailed. They are, in practice, regulations rather than guidelines. Because no guidelines or regulations can hope to anticipate, in detail, all experiments or circumstances, particularly in such a rapidly growing field, much time and effort are expended by investigators, RAC, and ORDA with requests to alter containment requirements or be granted exemptions from guidelines. The record shows that more often than not, after careful deliberation, the requests are granted.

Most scientists now conclude, after almost a decade of experience, deliberate risk assessment experimentation and theorizing, that the potential risks of recombinant DNA research have not materialized and most probably will not. While it is not possible to exclude with certainty risks to man or the ecosystem from application of recombinant DNA technology, at present these risks now appear to be much smaller than they appeared to be a few years ago.

In view of this conclusion, the current guidelines are too complex and restrictive of development of useful knowledge with direct health, agricultural, and economic benefits. They require a bureaucratic apparatus and an amount of paperwork that is viewed as excessive and excessively costly in terms of dollars and professionals' time.

2. *Abolish the Guidelines.*

a. *Advantages.* In the absence of known or suspected hazards, it appears unjustified to single out certain classes of experiments as requiring elevated levels or physical containment. Similarly unjustified is the expenditure of time and money required to maintain a regulatory apparatus that has been developed to protect society from hazards that may be non-existent. What residual uncertainties exist about such hazards can be handled more flexibly and more efficiently at the level of the individual scientist, with consultation with others as he sees fit. In some universities, the procedures for monitoring laboratory hazards will remain in force and provide a backup system for checking on the individual scientist. Other kinds of laboratory guidelines (e.g., those for working with human and animal pathogens) will still exist and will provide guidance for proper use of such organisms when recombinant DNA technology is used. Since many of the residual concerns are centered around changing the host range and pathogenicity of already existing pathogens, such appropriate containment procedures should be adequate and in line with the existing dangers.

b. *Disadvantages.* There remain some areas in which specific risks may exist. It would seem appropriate to have some mechanism for ensuring that scientists consider these risks before and during their experimentation, and that they seek some outside advice on how serious the risk may be. The current IBC structure, with ORDA and RAC as backup, provides such a structure. Its abolition will also leave the regulatory aspects of the field in chaos, and may encourage local jurisdictions, which may come to different conclusions about the degree of certainty concerning special risks, to enact less informed and less flexible guidelines and regulations for control of this field. In any case, individual determinations in many areas of the country will possibly require more time and effort of more scientists than if the same determination was made once centrally. The results will also be less uniform, encouraging pressure on local groups to keep containment low.

In the absence of a working procedure for monitoring other laboratory hazards, it seems foolhardy to abandon this mechanism even if one concludes that recombinant DNA risks are not qualitatively different from other research risks. It may be more appropriate to refine the mechanisms for tracking recombinant DNA experiments such that this mechanism can be used as a model for watching and preventing other research risks.

C. Recommendation

The Working Group on Revision of the Guidelines has proposed changes in the guidelines which (1) would significantly reduce required containment for a large class of experiments, (2) would employ other guidelines to set appropriate containment for working with pathogens containing recombinant DNA, and (3) would eliminate the prohibited class of experiments, including the large volume growth of recombinant DNA containing organisms, relegating them to the same status as other experiments. The proposal does retain, however, the requirement for IBC prereview of non-exempt experiments, and suggests that in specific cases where additional risk might be expected due to the nature of the recombinant DNA experiment itself, the containment should be adjusted accordingly. Thus, this procedure would have the effect of

(1) Supporting the notion that, for the vast majority of recombinant DNA experiments, the risks are those associated with the organisms involved. The appropriate containment for pathogens such as that specified by the proposed CDC Biosafety Guidelines for Microbiological and Biomedical Laboratories would continue to be enforced for experiments utilizing recombinant DNA.

(2) Simplifying the containment levels for the vast majority of experiments, and in most cases decreasing the required containment.

(3) Retaining flexibility in allowing change in containment in particular experiments where circumstances permit. Flexibility would reside primarily with the IBC, and, if necessary, questions could be referred to RAC.

(4) Maintaining the class of "exempt" experiments, which covers the majority of recombinant DNA experiments; new additions could be made to this class as justified.

(5) Maintaining prereview of all non-exempt experiments by the IBC. It is proposed that the requirements for the constitution of the IBC be dropped while the essential element of consultation with a group other than the principal investigator is retained.

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Appendix A of Annex B: Experiments with E. coli

One of the apprehensions of scientists early in the recombinant DNA era was that certain recombinant organisms (particularly *Escherichia coli*) might inadvertantly escape from the laboratory, particularly by way of inadvertant colonization of laboratory personnel. Furthermore it was feared that temporary intestinal colonization of a laboratory worker might result in exchange of genetic information from the laboratory *E. coli* strain to normal intestinal coliform flora. It was also feared by some that since some *E. coli* strains are pathogenic for man, perhaps *E. coli* K–12 should be regarded as a pathogen or "potential" pathogen.

Most of the early work with recombinant DNA involved *E. coli K–12.* Since the genetics of this strain are so well-studied, much (if not most) recombinant DNA research still involves derivatives of this strain. Thus it is

pertinent to discuss "risk" relevant to working with *E. coli* K–12.

A. History and Description of D. coli K–12

E. coli K–12 was first isolated from the intestine of a patient convalescing from diphtheria in Stanford University Hospital in the 1920's. Thereafter it was stored in the laboratory as a stock strain. Presumably, at the time of initial isolation it was a smooth strain adapted to colonization of the human colon. By the term smooth is meant the fact that the strain produces a complete lipopolysaccharide O antigen on its surface with many polymeric polysaccharide side chains. Over the course of years of laboratory passage *E. coli* K–12 has become irreversibly rough. By dint of its rough state, *E. coli* K–12 has lost a major prerequisite required for successful colonization of man and animals.

1. *E. coli as a Pathogen.* It is well-recognized that within the enormous species, *E. coli,* are strains that are true pathogens for man and animals. Depending on strain and host, certain *E. coli* can cause diarrheal illiness, dysentery, urinary tract infection or meningitis. While this is true, it is now known that such pathogenicity is *not* a general or even a potential feature of all *E. coli.* Rather, a constellation of specific virulence factors must be present in certain *E. coli* for them to be able to cause disease in man (or animals) and such virulence factors tend to be stable in only a relatively small number of strains or serotypes.

For example:

A. *E. coli meningitis*— Trauma or neurosurgical intervention which breaches the integrity of the meningeal coverings of the central nervous system can result in direct contamination and nosocomial infection with many gram-negative or gram-positive organisms. In the absence of the above, for all intents and purposes, primary *E. coli* meningitis is limited to infants less than three months of age; in fact most cases occur in neonates within 28 days of birth. In the early 1970's it was discovered that the *E. coli* that cause meningitis in infants have in common a surface capsular polysaccharide, K1, which confers virulence (*1,2*). By mid-infancy most individuals are already resistant to K1 encapsulated *E. coli.*

b. *Diarrheal Illness*—Certain *E. coli* can cause watery diarrhea. These strains, which include enterotoxigenic and enteropathogenic *E. coli,* possess a constellation of virulence properties which in tandem result in the ability to cause diarrhea. For example, enterotoxigenic *E. coli* that elaborate both heatlabile and heatstable enterotoxins usually also possess fimbrial colonization factors (adhesion pili) and such strains are usually limited to perhaps a dozen of the 164 *E. coli* O serogroups. Studies in pigs involving attempts to "create" a diarrheal pathogen by insertion of genes for adhesion pili (K88 antigen fimbriae) and enterotoxin into a K–12 strain were unsuccessful, (*3*) showing that the constellation of virulence properties of true porcine pathogens is more complex.

c. *Dysentery*—Certain strains of *E. coli* exist that are capable of causing a clinical syndrome identical to bacillary dysentery. Such strains are referred to as enteroinvasive and are limited to a very few *E. coli* O serogroups. Biochemically and serologically these strains bear many relationships to shigella.

d. *Urinary Tract Infections*—It is now recognized that the *E. coli* strains that are associated with upper urinary tract infections possess mannose resistant hemagglutinin, and fimbrial colonization factors that allow adhesion to epithelial cells of the urinary tract and prevent dislodgement by urine flow. Furthermore, it is currently recognized that urinary tract epithelial cells of certain individuals are more receptive than others to adhesion by *E. coli* strains possessing the fimbrial colonization factors.

2. *Inoculum Size and Mode of Infection*—It has been shown with volunteer studies that rather high inocula (10^6—10^{10}) and neutraliation of gastric acid (for example with $NaHCO_3$) are required to ensure colonization by enterotoxigenic *E. coli.* Volunteers with *E. coli* diarrheal disease did not transmit the infection by direct contact to uninoculated control volunteers with whom they were living in close quarters (*4*).

Volunteers challenged with enteropathogenic *E. coli* excreted large numbers of *E. coli* in thier stools and were colonized in their proximal small intestine but were not colonized in the throat (*5*).

The thrust of all these observations is that: *E. coli* enteropathogens are transmitted via large inocual contained within contaminated food and water vehicles. They are not spread by airborne or direct contact routes. In order to colonize the human gut, even with a known enteric pathogen, a large inoculum is required and modifications must be made (e.g., ingestion of $NaHCO_3$) to ensure survival of the *E. coli* through the gastric acid barrier. Thus, based on current knowledge and data it is extremely unlikely that individuals working in a recombinant DNA research laboratory could become colonized in their intestine by droplet, aerosol or contact transmission.

3. *Colonizability of Human Intestine by E. coli K–12: Summary of Feeding Studies*—Four groups have carried out studies in which high inocula (10^8—10^{10} organisms) of *E. coli* K–12 strains were fed to volunteers (*6–9*). The common observation from these studies is that *E. coli* K–12 is unable to colonize the human intestine. While counts of 10^5—10^7 K–12 organisms per gram of stool could be cultured on the first day postinfection, levels dropped to 10^2 or 10^3 by day 2 and K–12 was usually no longer detectable by day 5. In contrast, ingestion of 10^{10} organisms of a smooth non-pathogenic normal intestinal flora strain (*E. coli* HS) resulted in prolonged excretion of the organism (weeks) in high titer (10^6—10^8/gram stool), demonstrating clear-cut colonization (*8,10*).

There is only one report of apparent *in vivo* transmission of a conjugative plasmid from K–12 to normal host flora (*7*). In this instance, one day post-ingestion small numbers (10^1/gram stool) of resident coliforms were found to be carring the K–12 plasmid. The putative recombinants were detectable for only one day (*7*).

Feeding studies in volunteers have also been carried out with *E. coli* x1776, a highly defective, fastidious variant of K–12 that has been certified as host organism in EK2 levels of biological containment (*9*). x1776 was not detected in stools of volunteers following ingestion. When volunteers were fed x1776 containing plasmid pBR322, low levels of excreation, (10^1–10^2/gram for two days) were observed.

In summary, the above-mentioned feeding studies demonstrate that even after direct ingestion of large inocula, *E. coli* K–12 does not readily colonize the human intestine.

4. *Active Bacteriologic Surveillance for K–12 Colonization*—During a two year period fecal cultures were obtained every two to three days from laboratory workers handling nalidixic acid-resistant *E. coli* K–12 containing transmission-proficient R plasmids. These workers practiced no special precautions other than good microbiologic technique. Neither *E. coli* K–12 nor the R factors utilized in the laboratory were recovered from any stool culture at any time. These data demonstrate under "field conditions" that colonization of *E. coli* K–12 or acquisition by resident intestinal coliform flora of R factors used in the laboratory do not occur.

5. *"Non-Mobilizable Plasmids"*—Plasmid vectors have been created which are mobilized and transferred from bacterial cell to bacterial cell at a markedly reduced frequency than the parent strain (*12*). For example pBR322 is transferred approximately 100 thousand times less frequently than its colEl parent plasmid under comparable mating conditions. Risk assessment studies recently carried out at the Univiersity of Maryland School of Medicine under sponsorship of the National Institutes of Health have verified *in vivo* in man decreased mobilization of "nonmobilizable" plasmid vector pBR325 (*10*). Seventeen volunteers ingested 10^{10} *E. coli* HS (a smooth normal flora strain) containing plasmid pJBJK5 (a non-conjugative, mobilizable colEl variant containing tetracycline and chloramphenicol resistance genes). This strain heavily colonized the intestines of each of the 17 individuals, all of whom also ingested one gram of tetracycline daily. Normal flora recombinants containing plasmid pJBK5 were recovered from 9 to 17 volunteers. In contrast, when 15 volunteers ingested *E. coli* HS containing plasmid pBR325 (which also encodes for tetracycline resistance), although they also became heavily colonized while taking tetracyline daily, no resident flora recombinants were identified containing pBR325. These data attest to the decreased mobilizability in man of safe vector plasmids, even in the presence of antibiotic pressure modifying the environment in a way that recombinants would have enhanced survivability.

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Annex C—Minority Reports of Working Group on Revision of Recombinant DNA Guidelines

Further Recommendations

We the undersigned agree with the general thrust of the recommendations of the Working Group on Recombinant DNA Guidelines. In particular, we support the recommendation that those experiments, for which containment guidelines are retained, are to conform to good laboratory practice as described in the CDC and APHIS Quarantine rules. The recommendation that abolishes the category of prohibited experiments is scientifically sound in the light of current knowledge. The content of the suggested containment guidelines follows from the overall conclusion that research with recombinant DNA imposes no more risk than any other biological research. The background paper amply documents this point.

Therefore, we find no reason to retain the Administrative Guidelines (Section IV). The spirit of the Baltimore-Campbell resolution and, as titled, its major purpose, was to convert the NIH Regulations into Guidelines. Retaining pages of bureaucratic regulations for what is now perceived to require only limited oversight is a waste of time and effort, will not add to safety in any way and will be disdained as bureaucratic capriciousness.

Edward A. Adelberg, Kenneth I. Berns, Herman W. Lewis, Sue A. Tolin, Norton D. Zinder.

Given the recommendations for the containment guidelines and what would be proper for the administrative guidelines, we the undersigned conclude that special guidelines for recombinant DNA are totally unnecessary. Therefore, we further recommend that as of June 23, 1982 the NIH Guidelines cease to exist.

The arguments for their retention are primarily social and political; they do not have a scientific base (see Background Paper). It seems right, at this time, to disavow such arguments and base our conclusions solely on the scientific issues. RAC should recommend the abolition of the Guidelines. In doing so, it should prepare a strong statement of its rationale to be published in *Science* and *Nature* as well as the **Federal Register.** This statement should also contain as recommendation the containment guidelines for recombinant DNA experiments developed by the Working Group. Prepared as the culmination of years of consideration and analysis, such a statement would serve to inform both the scientific community and the public of the safety of research with recombinant DMA.

Edward A. Adelberg, Norton D. Zinder.

Annex D—Draft Minutes of Relevant Portion of September 10–11, 1981 RAC Meeting

Drs. David Baltimore and Allan Campbell, RAC members, had proposed a major revision of the Guidelines (Baltimore-Campbell proposal) which was considered by the RAC at its April 1981 meeting. At the April 1981 meeting, a Working Group on Revision of the Guidelines was established to review the Baltimore-Campbell proposal as well as other approaches which might lead to a major revision of the Guidelines. The Working Group met on June 1, 1981, and on July 9, 1981. The Working Group prepared a proposal for revising the Guidelines, a summary of its actions, and a document entitled "Evaluation of the Risks Associated with Recombinant DNA Research." Two minority reports were prepared by several members of the Working Group. The Working Group report and the minority reports were distributed to RAC members prior to the September 1981 meeting.

Mr. Thornton asked Dr. Gottesman to introduce the Working Group's report. Dr. Gottesman reviewed the highlights of the report. She noted that the revision of the Guidelines promulgated on July 1, 1981, already exempts many experiments in three major host-vector systems. The Baltimore-Campbell proposal would convert mandatory Guidelines to a voluntary code of good practice and would set as containment levels those appropriate for the organism being used. The Working Group considered various approaches; the majority supported a proposal which adopts the containment provisions of the Baltimore-Campbell proposal but retains the mandatory aspect of the Guidelines. The proposal has not yet been published as a proposed major

action in the **Federal Register.** The RAC may wish to modify the proposal before its formal publication in the **Federal Register** for public comment.

Dr. Gottesman noted that the background document discusses basic assumptions. It is difficult to imagine hazards resulting from random combinations of DNA. Furthermore, deliberate combinations will not be harmful in most cases. However, there are still some questions about certain experiments. The issue is how to deal with the latter experiments. The proposal of the Working Group would retain IBC prereview so that there is a level of review beyond the investigator.

Dr. Gottesman then reviewed the main points of the Working Group proposal. The proposed containment levels are very similar to those of the Baltimore-Campbell proposal, i.e., containment would be largely based on the pathogenicity of the host. For all non-exempt experiments, at least the P1 level would be recommended. The Working Group proposal eliminates reference to biological containment in Part III of the Guidelines. The Working Group proposal also adds in admonition which reads as follows:

> If there is clear evidence that the donor DNA will significantly change the pathogenicity of the host, the containment level appropriate to the anticipated change will be applied.

While the Baltimore-Campbell proposal would be a voluntary code of practice, the Working Group proposal retains IBC prereview of covered experiments and retains Section IV–G of the Guidelines which discusses possible penalties for failing to follow the Guidelines. However, the Working Group recommends eliminating membership requirements for IBCs currently specified in Section IV–D of the Guidelines. While the Baltimore-Campbell proposal retains the prohibition section of the Guidelines (I–D), the Working Group proposal eliminates the prohibitions on the basis that currently prohibited experiments would be prereviewed by an IBC in their proposal. Dr. Gottesman said that she felt that the major issues for discussion are: prohibitions, prereview of covered experiments, and containment levels.

Dr. Berns noted that five Working Group members, including himself, submitted a minority report which disagrees with the Working Group's proposal to retain the mandatory nature of the Guidelines. The minority report recommends elimination of Part IV of the Guidelines.

Dr. Goldstein said that he agrees with parts of the Working Group report. He said that IBCs are still needed although a survey in California has indicated great variation in IBCs. He also said that prohibited experiments need to be more clearly considered.

Dr. Harris said that she felt that the Working Group's report is an acceptable compromise.

Ms. King said that the report suggests a direction for movement but that details need to be worked out. She said that if there are Guidelines, there should be sanctions. She said that distinguishing NIH grantees from non-grantees is not unique; she cited the situation with human experimentation. Ms. King said that she strongly favors retention of the sanctions.

Dr. Nightingale said that she supports the recommendations of the Working Group, saying that they represent a good compromise. She expressed concern about the availability of other guidelines cited in Working Group report. In this regard, she noted the many comments received on the proposed CDC Biosafety Guidelines for Microbiological and Biomedical Laboratories. She also expressed concern that prohibition I–D–4, dealing with deliberate release into the environment, had not been dealt with. Dr. Nightingale said there is a necessity for accountability when public funds are being used. She said the RAC needs an assessment of outside perceptions and that the background document needs further work.

Dr. Zinder then addressed the RAC. He noted that he had prepared the minority reports to the Working Group's recommendations. He said that although there is a disagreement about the administrative aspects of the Guidelines, there was unanimity in the Working Group in favor of the new proposed containment levels. He noted that although inclusion of a slightly modified Part IV was recommended by the Working Group, five members have now endorsed a minority report stating that Part IV should be removed from the Guidelines. Drs. Adelberg and Zinder also signed a second minority report recommending complete elimination of the Guidelines. Dr. Zinder said that he and the scientists concerned about recombinant DNA originally proposed guidelines which would give guidance and not be enforced, rather than rules. He cited some of the history leading to the issuance of mandatory guidelines in 1976. Dr. Zinder said that if Part IV is retained in the revised Guidelines, there should be a strong justification for retaining it stated in a position paper. He said that he prefers that the Guidelines be rescinded and replaced with a simple recommendation. He said that if scientists are to be encouraged to speak up in the future about conjectural risks, they must be shown that when interim regulation is subsequently shown to be superfluous, it can be removed.

Dr. Baltimore said the Baltimore-Campbell proposal was a compromise between scientific judgment that there is no justification for Guidelines being other than a code of accepted practice, and the necessity for considering political and social factors. Their proposal retained the prohibitions, which he said are one of the most noted parts of the Guidelines. It has been argued that the RAC should not consider political and social factors; however, Dr. Baltimore did not agree. He said that the prohibitions have less and less justification and that he finds no difficulty in accepting the Working Group's recommendation regarding elimination of prohibited experiments, except for elimination of the prohibition against acquisition of a drug resistance trait in those cases in which such acquisition could compromise the use of a drug to control disease agents in human or veterinary medicine or agriculture. Concerned scientists originally proposed guidelines meaning only guidance and not regulations; the RAC will have to decide the future course of the Guidelines. He noted that there apparently already is general agreement in the RAC on modifying Part III dealing with containment levels and agreement on retaining the exemptions. The issue is Part IV of the Guidelines. He said that he feels that it is anomalous to retain the current procedures section of the Guidelines. However, political and social issues need to be considered. If the Federal government pulled completely out of the issue, local governments might well overreact. The Federal government needs to provide surveillance, a forum for considering questions, and an office where inquiries can be authoritatively answered. Therefore, maintenance of the RAC, ORDA, and abbreviated guidelines are necessary. Dr. Baltimore expressed support for the original Baltimore-Campbell proposal, with some modifications based on the Working Group recommendations. Dr. Zinder said that if Guidelines are to be retained, it is for political and social reasons. However, the political climate has changed. In New York State, which previously passed a law regulating recombinant DNA research, a bill has been introduced to repeal the law based on the assertion that the medical institutions which perform such research in New York State have proven to be trustworthy.

Dr. Nightingale said that she agrees with most of what Dr. Baltimore said. In order to implement sound public policy there are three basic ingredients: knowledge base, commitment of leadership, and appropriate social strategy. She said that in this case there is much agreement on the knowledge base and that there is need for change and the direction of that change. There is more disagreement on the appropriate social strategy. Having Federal involvement is one method of tempering local extremism.

Ms. King noted that the Baltimore-Campbell proposal would put industry and academia on an equal footing. She said that the RAC should consider a special meeting and public hearing on the proposed changes.

Dr. Gottesman noted that the current Guidelines are flexible and that the vast majority of experiments done today are exempt. She feels that there are scientific reasons for having a group other than the principal investigator look at the experiments still covered by the Guidelines; they should be reviewed by IBCs. She said that the critical issues in Part IV of the Guidelines are IBC prereview and compliance. She said that the Working Group's proposal does not involve an immense administrative burden.

Dr. Goldstein said that he also shares concerns about the scientific issues. He noted the number of new companies becoming involved in recombinant DNA research.

Dr. Brill stated that at a recent public meeting regarding a genetic engineering company in Madison, Wisconsin, no concerns were expressed about use of recombinant DNA technology.

Dr. McKinney said that the handling of the prohibitions is an important issue. They could perhaps be changed to cautionary advisories. He also said that citation of CDC and USDA guidelines needs to be considered. He preferred that NIH retain some form of guidance over recombinant DNA research.

With regard to the proposed revision of the CDC guidelines, Dr. Berns said that he expects great improvement in the document in the near future. He had discussed proposed revisions of the NIH Guidelines at a recent Gordon conference; most scientists there did not favor complete abolition of the Guidelines and favored instead something like the Baltimore-Campbell proposal. He also cited his local Congressman's concern about the potential for increasing public concern about recombinant DNA technology. It is important in the Guidelines revision that public confidence be maintained.

Dr. Zinder said that the public trusts academic researchers, but not industry.

Dr. Gottesman stressed that under the Working Group's proposal, the IBC would make the decision on containment using the CDC document only as guidance. Dr. Goldstein said that how the IBCs use and interpret the CDC guidelines should be made more explicit, so that there are uniform standards.

Dr. McGarrity said that he is comfortable in accepting voluntary guidelines. He felt that the Working Group's background report is excellent and that perhaps an abridged version could be published for educating the general public.

Dr. Holmes said that he supports retaining the current prohibition dealing with the introduction of drug resistance traits. He said that to make the Guidelines voluntary would be a mistake and could invite legislation.

Dr. Baltimore said that the concern raised in the Boston area is not a unique situation and that activities at the Federal level are still important. He said that since there are differences in the science done at different institutions it is not suprising, and not relevant to the present discussion, that different IBCs in California operate differently. Dr. Baltimore emphasized his view that IBC prereview is a serious obstruction of science, which results in scientific momentum being lost.

Dr. McKinney pointed out that the NIH could still choose to mandate guidelines even if the RAC recommends otherwise. He suggested that reference to CDC and USDA guidelines not be incorporated into the body of the text of the revised guidelines; rather they could be cited as references.

Ms. King suggested that the RAC should structure the issues on which it wants public comment, such as treatment of prohibitions and the voluntary vs. mandatory nature of the guidelines. Dr. Talbot pointed out that the RAC could follow Ms. King's suggestion and present issues for public comment. The alternative would be for the RAC to accept the Working Group proposal, the Baltimore-Campbell proposal, or an amalgam of the two. Following the meeting, NIH staff could then develop a new version of the Guidelines based on the RAC proposal and put this out for public comment.

Dr. Harris then moved to accept the report of the Working Group so that discussion could proceed to consider the report section by section. Dr. Mason seconded the motion. There followed discussion of the effect of such a motion.

Dr. Ahmed praised the report of the Working Group. He favored publication for public comment of a series of different options. Dr. Mason expressed concern about eliminating all of the prohibitions. Dr. Saginor suggested that the RAC might first consider the Adelberg-Zinder minority proposal to abolish the Guidelines. Dr. McKinney said that he considered the Working Group's recommendations as too cursory. Dr. Gottesman responded that the Working Group had considered the issues in-depth at two meetings and had prepared a report on its evalution of the risks associated with recombinant DNA research. The RAC could change or elaborate on the recommendations before seeking public comment. Dr. Zinder said that the recommendations of the Working Group were adopted unanimously except for those concerning Part IV of the Guidelines.

Dr. Baltimore, in the interests of providing a forum for RAC discussion of the points of difference between the various proposals, moved a seven part motion as a substitute for Dr. Harris' motion:

1. Accept the first section of the Baltimore-Campbell proposal, as follows:

"Section I-A of the NIH Guidelines will be replaced with the following:

'I-A. *Purpose.* The purpose of these Guidelines is to specify standard practices for constructing and handling (i) recombinant DNA molecules and (ii) organisms and viruses containing recombinant DNA molecules. Adherence to these standards by all laboratories using recombinant DNA is recommended.' "

2. Accept the second section of the Baltimore-Campbell proposal, as follows:

"Part I-C of the NIH Guidelines shall be eliminated."

3. Accept the second section of the Working Group proposal, as follows:

"Section I-D of the Guidelines, *Prohibitions,* would be eliminated."

4. Accept the third section of the Working Group report modified by removing references to CDC Guidelines and USDA Regulations and treating these references in a footnote, as follows:

"Part III of the Guidelines would be replaced with the following language:

'Part III discusses experiments covered by the Guidelines. The reader should first consult Part I, where exempt experiments are listed.

'Where recommended physical containment levels applicable to non-recombinant DNA experiments exist for either the host or the vector,* recombinant DNA experiments should be carried out at

*Such as those specified by CDC Guidelines or the USDA Quarantine Regulations.

containment levels at least as high as those recommended for non-recombinant DNA experiments. If there is clear evidence that the donor DNA will significantly change the pathogenicity of the host, the containment level appropriate to the anticipated change will be applied. Otherwise, all experiments may be carried out under conditions of P1 or P1–LS physical containment.'"

5. The following admonition would be added:

"No experiments should be performed which involve 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 a drug to control disease agents in human or veterinary medicine or agriculture."

6. Accept the fourth section of the Baltimore-Campbell proposal, as follows:

"Part IV of the NIH Guidelines shall be eliminated, with the following exceptions:

"(a) Those definitions listed in Part IV–C which may be needed to clarify statements made elsewhere in the Guidelines shall be retained.

"(b) Those portions of Part IV–E defining the composition of RAC and prescribing rules for RAC procedures shall be retained.

"(c) The following statement shall be added:

"Each institution conducting or sponsoring recombinant DNA research should take responsbility for monitoring its own activities in this area. Any unusual events that might be associated with the use of recombinant DNA molecules should be reported to the Director, NIH."

7. Accept the fifth section of the Baltimore-Campbell proposal with deletion of the words "submitted in support of requests for exceptions from the prohibitions," as follows:

"Section VI of the Guidelines will be eliminated, except for those portions of Section VI–F relevant to the protection of proprietary information."

Dr. Berns seconded the motion.

Dr. Saginor suggested an amendment to Dr. Harris' motion in the form of a policy statement that there is a continuing need for the RAC and applicable recombinant DNA guidelines. The purpose of the amendment was to indicate that the Adelberg-Zinder proposal is not being accepted. Dr. Harris agreed to the amendment.

Ms. King said she wanted the RAC to vote on replacing parts 1 and 6 of the Baltimore motion with wording from the Working Group proposal. It was suggested that votes be on one part at a time. Ms. King then moved to replace the first part of Dr. Baltimore's motion with the first section of the Working Group's proposal as follows:

"Section I–A of the Guidelines would be amended to read as follows:

"I–A. *Purpose.* The purpose of these Guidelines is to specify standard practices for constructing and handling (i) recombinant DNA molecules and (ii) organisms and viruses containing recombinant DNA molecules."

The motion was seconded by Dr. Goldstein. Ms. King stated that she favors retention of limited Guidelines that require IBC review, and she favors an oversight function for the RAC; she does not support self-regulation. Dr. Baltimore did not accept Ms. King's proposed amedment. Dr. Berns pointed out that the substitution Ms. King was proposing did not make much difference. The real point of contention in the RAC concerned part six of Dr. Baltimore's motion.

Ms. King withdrew her previous motion and then moved to delete part six of Dr. Baltimore's motion. If her motion were accepted, this would leave intact Part IV of the Guidelines. It was pointed out that the Working Group had proposed a change in Part IV dealing with IBC membership. Ms. King said that if her motion passed, then another perfecting motion could be introduced dealing with IBC membership. Dr. Harris seconded. The motion failed to carry by a vote of nine in favor, twelve opposed, with no abstentions.

Dr. Fedoroff noted that the motion as it stands would eliminate all prohibitions including the prohibition against deliberate release into the environment. Dr. Baltimore suggested that if the RAC wished, a statement regarding deliberate release could be included with the admonition on drug resistance. Dr. Berns said that in his view the recommendation that experiments be conducted under P1 containment precludes deliberate release into the environment.

Dr. Maas then moved to add the current prohibition on the cloning of certain toxins to the admonition on drug resistance. Dr. Goldstein seconded. Dr. Gottesman said that the cloning of toxins is an example of an area of concern. She noted that the RAC Working Group on Toxins recommended at the last RAC meeting prohibition of cloning of certain toxin genes and that other experiments involving cloning of toxin genes should proceed only in *E. coli* K–12 in the absence of special review by ORDA. Dr. Baltimore agreed to accept addition of the wording regarding toxins currently in Section I–D–2 to the admonition on drug resistance and to retain Appendix G of the current Guidelines.

Dr. Ahmed moved that a working group be appointed to study the prohibitions and report back to the RAC. Dr. Goldstein seconded the motion. Dr. Mason disagreed, noting that at the last meeting a working group had been appointed to report on revision of the Guidelines. They had reported, and now the RAC was working through the proposal to prepare material for public comment. The motion failed to carry by a vote of three in favor, fourteen opposed, with three abstentions.

Mr. Thornton recognized Dr. Susan Wright. She said the RAC was short-circuiting long and detailed discussions it should have on all the critical issues. She asked RAC members to acknowledge ties that they might have with genetic engineering companies. She said there should be discussion of why the working group had decided to eliminate public members on IBCs. She expressed concern about the currently prohibited experiments and large-scale experiments. She cited a report she had submitted, prepared for the Commission of the European Communities, entitled "Hazards Involved in the Industrial Use of Micro-organisms." She said that change of phenotype due to mutation and discharge of waste into the environment are important issues among many others that need to be considered before a decision is reached.

Dr. Fedoroff said that there should be flexibility to have a group look at and approve specific experiments which are otherwise admonished against. Dr. Baltimore said investigators wishing to do such experiments could come to the local IBC or the RAC to discuss conditions under which such experiments could be done.

Dr. Berns said that at a meeting of the Large Scale Review Working Group on September 9, 1981, none of the members thought that the large-scale prohibition should be retained.

Mr. Thornton recognized Ms. Claire Nader who said that the RAC should look at the assumptions behind the recommendations such as that all corporations will do the right thing, and that the technology is safe. She said that there were no experts on corporate behavior, or law enforcement, or anti-trust questions on the RAC. She said the RAC should have on it people who want to talk about risks. She criticized the way in which the RAC was proceeding.

Dr. Nightingale said that a working group on the prohibitions was appointed over a year ago and that the prohibitions have been discussed extensively before this meeting. Dr. Gottesman said that it was peculiar to be concerned about the prohibitions and at the same time recommending that the entire system become voluntary. She said that perhaps

there could be a recommendation that these experiments be reviewed by the RAC.

Mr. Daloz moved that a vote be taken on Dr. Baltimore's motion, as amended. The motion to end discussion and vote failed to carry by a vote of four in favor, fourteen opposed, with three abstentions.

Dr. Ahmed said he wanted detailed procedures built into the revised Guidelines for handling the currently prohibited experiments. Dr. Baltimore said that the absence of detailed procedures pertains in the case of all nonrecombinant DNA laboratory work including that with known pathogens.

Mr. Thornton asked for a show of hands of RAC members who wished to continue discussion of this agenda item for an additional thirty minutes until approximately 3:30 p.m. The vote was eighteen in favor, one opposed.

Dr. Mason said that the RAC and the Guidelines cannot deal with scientists or industrial groups who are uninformed, dishonest, or careless. We have tried to produce guidelines that reponsible people will follow. There is no way to provide for every contingency.

Dr. Holmes moved to add current prohibition I–D–4 ("deliberate release into the environment of any organism containing recombinant DNA.") to the admonitions regarding cloning of toxins and transfer of drug resistance traits. Dr. Landy supported inclusion of I–D–4; Dr. Berns did not support it. The motion failed to carry by a vote of eight in favor, ten opposed, with two absentions.

Dr. Baltimore's amended motion was reviewed. Dr. Talbot said that if the proposal passed, the NIH staff would prepare a version of proposed revised Guidelines based on the proposal, and that it would be put in the **Federal Register** for public comment, along with background describing the work of the working group and the deliberations of the RAC. NIH would actively solicit comment on the proposal beyond its publication in the **Federal Register.**

The question was called and the vote to substitute Dr. Baltimore's motion, as amended, for Dr. Harris' motion was fifteen in favor, three opposed, with two abstentions. Dr. Ahmed asked to be recorded as voting against the motion. The motion was as follows:

"1. Section I–A of the Guidelines would be amended to read as follows:

"I–A. Purpose. The purpose of these Guidelines is to specify standard practices for constructing and handling (i) recombinant DNA molecules and (ii) organisms and viruses containing recombinant DNA molecules. Adherence to these standards by all laboratories using recombinant DNA is recommended.

"2. Section I–C of the Guidelines would be eliminated.

"3. Section I–D of the Guidelines, *Prohibitions,* would be eliminated.

"4. Part III of the Guidelines would be replaced with the following language:

"Part III discusses experiments covered by the Guidelines. The reader should first consult Part I, where exempt experiments are listed.

"Where recommended physical containment levels applicable to non-recombinant DNA experiments exist for either the host or the vector*, recombinant DNA experiments should be carried out at containment levels at least as high as those recommended for non-recombinant DNA experiments. If there is clear evidence that the donor DNA will significantly change the pathogenicity of the host, the containment level appropriate to the anticipated change will be applied. Otherwise, all experiments may be carried out under conditions of P1 or P1–LS physical containment.

"5. Material would be added to Part III, as follows:

"No experiments should be performed which involve:

"(a) Deliberate transfer of a drug resistance trait to micro-organisms that are not known to acquire it naturally, if such acquisition could compromise the use of a drug to control disease agents in human or veterinary medicine or agriculture.

"(b) Deliberate formation of recombinant DNAs containing genes for the biosynthesis of toxins lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight (e.g., the botulinum toxins, tetanus toxin, diphtheria toxin, *Shigella dysenteriae* neurotoxin), Guidelines for the cloning of DNAs containing genes coding for the biosynthesis of toxins which are lethal to vertebrates at 100 nanograms to 100 micrograms per kilogram body weight are specified in Appendix G.

"6. Part IV of the Guidelines would be eliminated with the following exceptions:

"(a) Those definitions listed in Part IV–C which may be needed to clarify statements made elsewhere in the Guidelines shall be retained.

"(b) Those portions of Part IV–E defining the composition of RAC and prescribing rules for RAC procedures shall be retained.

"(c) The following statement shall be added:

"Each institution conducting or sponsoring recombinant DNA research should take responsibility for monitoring its own activities in this area. Any unusual events that might be associated with the use of recombinant DNA molecules should be reported to the Director, NIH.

"7. Section VI of the Guidelines will be eliminated, except for those portions of Section VI–F relevant to the protection of proprietary information."

The vote on this substitute motion was called, and the vote was sixteen in favor, three opposed, with one abstention.

* Such as those specified by the CDC Guidelines or the USDA Quarantine Regulations.

Dr. Zinder requested that a motion be introduced in support of the Adelberg-Zinder proposal to eliminate the Guidelines and the RAC. No motion was introduced.

Annex E

Current Guidelines for Research Involving Recombinant DNA Molecules

October 1981.

These Guidelines are the Guidelines as published in the **Federal Register** of July 1, 1981 (46 FR 34462), with the incorporation of those changes promulgated in the **Federal Register** of October 30, 1981, Part V.

Table of Contents

I. Scope of the Guidelines

I–A. *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.

I–B. *Definition of Recombinant DNA Molecules.* In the context of these Guidelines, recombinant DNA molecules are defined as either (i) 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 (i) above.

I–C. *General Applicability.* See Section IV–B.

I–D. *Prohibitions.* The following experiments are not to be initiated at the present time:

I–D–1. Formation of recombinant DNAs derived from the pathogenic organisms classifed [1] as Class 4 or 5 [2] or from cells known [2A] to be infected with such agents, regardless of the host-vector system used.

I–D–2. Deliberate formation of recombinant DNAs containing genes for the biosynthesis of toxins lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight (e.g., the botulinum toxins, tetanus toxin, diphtheria toxin, *Shigella dysenteriae* neurotoxin). Guidelines for the cloning of DNAs containing genes coding for the biosynthesis of toxins which are lethal to vertebrates at 100 nanograms to 100 micrograms per kilogram body weight are specified in Appendix G, which overerides other parts of the Guidelines (e.g., exemptions, return to host of origin, etc.).

I–D–3. [Deleted].

I–D–4. Deliberate release into the environment of any organism containing recombinant DNA

I–D–5. 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 a drug to control disease agents in human or veterinary medicine or agriculture. [2A]

I–D–6. Large-scale experiments (e.g., more than 10 liters of culture) with organisms containing recombinant DNAs other than those listed in Appendix C, Section 2, 3, and 4 of the Guidelines, unless the recombinant DNAs are rigorously characterized and the absence of harmful sequences established [3]. (See Section IV–E–1–b–(3)–(d).)

I–D (1–6). Experiments in Categories I–D–1 to I–D–6 may be excepted [4] from the prohibitions (and will at that time be assigned appropriate levels of physical and biological containment) provide that these experiments are expressly approved by the Director, National Institutes of Health (NIH), with advice of the Recombinant DNA Advisory Committee (RAC), after appropriate notice and opportunity for public comment. (See Section IV–E–1–b–(1)–(e).)

Experiments in Categories I–D–1, I–D–2, I–D–5, and experiments involving "wild type" host-vector systems are excepted from the prohibitions, provided that these experiments are designed for risk-assessment purposes and are conducted within the NIH high-containment facilities located in Building 41–T on the Bethesda campus and in Building 550 located at the Frederick Cancer Research Center. The selection of laboratory practices and containment equipment for such experiments shall be approved by the Office of Recombinant DNA Activities (ORDA) following consultation with the RAC Risk Assessment Subcommittee and the NIH Biosafety Committee. ORDA shall inform RAC members of the proposed risk-assessment projects at the same time it seks consultation from the RAC Risk Assessment Subcommittee and the NIH Biosafety Committee. If a major biohazard is determined, the clones will be destroyed after the completion of the experiment rather than retaining them in the high containment facility. Other clones that are non-hazardous or not of major hazard will be retained in the high containment.

I–E. *Exemptions.* It must be emphasized that the following exemptions [4] are not meant to apply to experiments described in the Sections I–D–1 to I–D–5 as being prohibited. In addition, any recombinant DNA molecules involving DNA from Class 3 organisms [1] or cells known to be infected with these agents, or any recombinant DNA molecules which increase the virulence and host-range of a plant pathogen beyond that which occurs by natural genetic exchange, are not exempt unless specifically so designated by NIH under Section I–E–5. Also, Appendix G overrides the exemptions for specified experiments involving genes coding for toxins.

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

I–E–1. Those that are not in organisms or viruses. [5]

I–E–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.

I–E–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 a 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).

I–E–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. A list of such exchanges will be prepared and periodically revised by the Director, NIH, with advice of the RAC, after appropriate notice and opportunity for public comment. (See Section IV–E–1–b–(1)–(d).) Certain classes are exempt as of publication of these Revised Guidelines. The list is in Appendix A. An updated list may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20205.

I–E–5. Other classes of recombinant DNA molecules, if the Director, NIH, 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. (See Section IV–E–1–b–(1)–(d).) Certain classes are exempt as of publication of these Revised Guidelines. The list is in Appendix C. An updated list may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland, 20205.

I–F. *General Definitions.* See Section IV–C.

II. 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. [6–19] 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.

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 here in order that such combinations can be conveniently expressed in the Guidelines.

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 that indicated here without affecting risk. Indeed, we urge that individual investigators device simple and more effective containment procedures and that investigators and institutional biosafety committees recommend changes in the Guidelines to permit their use.

II–A. *Standard Practices and Training.* The first principle of containment is a strict adherence to good microbiological practices. [6–15] Consequently, all personnel directly or indirectly involved in experiments on recombinant DNAs must receive adequate instruction. (see Sections IV–D–1–g, IV–D–5–d and IV–D–8–b.). 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 principal investigator must ensure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan. (See Sections IV–D–5–e and IV–D–3–d.) If a research group is working with a known pathogen where there is an effective vaccine it should be made available to all workers. Where serological monitoring is clearly appropriate it shall be provided. (See Sections IV–D–1–h and IV–D–8–c.)

II–B. *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 P1, P2, P3, and P4, 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. For example, the "Classification of Etiologic Agents on the Basis of Hazard,"[7] prepared by the Centers for Disease Control, describes four general levels which roughly correspond to our descriptions for P1, P2, P3, and P4; and the National Cancer Institute describes three levels for research on oncogenic viruses which roughly correspond to our P2, P3, and P4 levels.[8]

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 P3 and P4 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. Consideration will also be given by the Director, NIH, with the advice of the Recombinant DNA Advisory Committee to other combinations which achieve an equivalent level of containment. (See Section IV-E-1-b-(2)-(b).) Additional material on physical containment for plant host-vector systems is found in Sections III-C-3 and III-C-4.

II-B-1. *P1 Level.*

II-B-1-a. *Laboratory Practices.*

II-B-1-a. Laboratory doors shall be kept closed while experiments are in progress.

II-B-1-a-(2). Work surfaces shall be decontaminated daily, and immediately following spills of organisms containing recombinant DNA molecules.

II-B-1-a-(3). All biological wastes shall be decontaminated before disposal. Other contaminated materials, such as glassware, animal cages, and laboratory equipment, shall be decontaminated before washing, reuse, or disposal.

II-B-1-a-(4). Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

II-B-1-a-(5). Eating, drinking, smoking, and storage of foods are not permitted in the laboratory area in which recombinant DNA materials are handled.

II-B-1-a-(6). Persons shall wash their hands after handling organisms containing recombinant DNA molecules and when they leave the laboratory.

II-B-1-a-(7). Care shall be taken in the conduct of all procedures to minimize the creation of aerosols.

II-B-1-a-(8). Contaminated materials that are to be decontaminated at a site away from the laboratory shall be placed in a durable leak-proof container, which is closed before removal from the laboratory.

II-B-1-a-(9). An insect and rodent control program shall be instituted.

II-B-1-a-(10). The use of laboratory gowns, coats, or uniforms is discretionary with the laboratory supervisor.

II-B-1-a-(11). Use of the hypodermic needle and syringe shall be avoided when alternative methods are available.

II-B-1-a-(12). The laboratory shall be kept neat and clean.

II-B-1-b. *Containment Equipment.* Special containment equipment is not required at the P1 level.

II-B-1-c. *Special Laboratory Design.* Special laboratory design is not required at the P1 level.

II-B-2. *P2 Level.*

II-B-2-a. *Laboratory Practices.*

II-B-2-a-(1). Laboratory doors shall be kept closed while experiments are in progress.

II-B-2-a-(2). Work surfaces shall be decontaminated daily, and immediately following spills of organisms containing recombinant DNA molecules.

II-B-2-a-(3). All laboratory wastes shall be steam-sterilized (autoclaved) before disposal. Other contaminated materials such as glassware, animal cages, laboratory equipment, and radioactive wastes shall be decontaminated by a means demonstrated to be effective before washing, reuse, or disposal.

II-B-2-a-(4). Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

II-B-2-a-(5). Eating, drinking, smoking, and storage of food are not permitted in the laboratory area in which recombinant DNA materials are handled.

II-B-2-a-(6). Persons shall wash their hands after handling organisms containing recombinant DNA molecules and when they leave the laboratory.

II-B-2-a-(7). Care shall be exercised to minimize the creation of aerosols. For example, manipulations such as inserting a hot inoculating loop or needle into a culture, flaming an inoculation loop or needle so that it splatters, and forceful ejection of fluids from pipettes or syringes shall be avoided.

II-B-2-a-(8). Contaminated materials that are to be steam sterilized (autoclaved) or decontaminated at a site away from the laboratory shall be placed in a durable leak-proof container, which is closed before removal from the laboratory.

II-B-2-a-(9). Only persons who have been advised of the nature of the research being conducted shall enter the laboratory.

II-B-2-a-(10). The universal biohazard sign shall be posted on all laboratory access doors when experiments requiring P2 containment are in progress. Freezers and refrigerators or other units used to store organisms containing recombinant DNA molecules shall also be posted with the universal biohazard sign.

II-B-2-a-(11). An insect and rodent control program shall be instituted.

II-B-2-a-(12). The use of laboratory gowns, coats, or uniforms is required. Laboratory clothing shall not be worn to the lunch room or outside of the building in which the laboratory is located.

II-B-2-a-(13). Animals not related to the experiment shall not be permitted in the laboratory.

II-B-2-a-(14). Use of the hypodermic needle and syringe shall be avoided when alternative methods are available.

II-B-2-a-(15). The laboratory shall be kept neat and clean.

II-B-2-a-(16). Experiments of lesser biohazard potential can be carried out concurrently in carefully demarcated areas of the same laboratory.

II-B-2-b. *Containment Equipment.* Biological safety cabinets[20] shall be used to contain aerosol-producing equipment, such as blenders, lyophilizers, sonicators, and centrifuges, when used to process organisms containing recombinant DNA molecules, except where equipment design provides for containment of the potential aerosol. For example, a centrifuge may be operated in the open if a sealed head or safety centrifuge cups are used.

II-B-2-c. *Special Laboratory Design.* An autoclave for sterilization of wastes and contaminated materials shall be available in the same building in which organisms containing recombinant DNA molecules are used.

II-B-3. *P3 Level.*

II-B-3-a. *Laboratory Practices.*

II-B-3-a-(1). Laboratory doors shall be kept closed while experiments are in progress.

II-B-3-a-(2). Work surfaces shall be decontaminated following the completion of the experimental activity, and immediately following spills of organisms containing recombinant DNA molecules.

II-B-3-a-(3). All laboratory wastes shall be steam-sterilized (autoclaved) before disposal. Other contaminated materials, such as glassware, animal cages, laboratory equipment, and radioactive wastes, shall be decontaminated by a method demonstrated to be effective before washing, reuse, or disposal.

II-B-3-a-(4). Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

II-B-3-a-(5). Eating, drinking, smoking, and storage of food are not permitted in the laboratory area in which recombinant DNA materials are handled.

II-B-3-a-(6). Persons shall wash their hands after handling organisms containing recombinant DNA molecules and when they leave the laboratory.

II-B-3-a-(7). Care shall be exercised to minimize the creation of aerosols. For example, manipulations such as inserting a hot inoculating loop or needle into a culture, flaming an inoculation loop or needle so that it splatters, and forceful ejection of fluids from pipettes or syringes shall be avoided.

II-B-3-a-(8). Contaminated materials that are to be steam-sterilized (autoclaved) or decontaminated at a site away from the laboratory shall be placed in a durable leak-proof container, which is closed before removal from the laboratory.

II-B-3-a-(9). Entry into the laboratory shall be through a controlled access area. Only persons who have been advised of the nature of the research being conducted shall enter the controlled access area. Only persons required on the basis of program or support needs shall be authorized to enter the laboratory. Such persons shall be advised of the nature of the research being conducted before entry, and shall comply with all required entry and exit procedures.

II-B-3-a-(10). Persons under 16 years of age shall not enter the laboratory.

II-B-3-a-(11). The universal biohazard sign shall be posted on the controlled access area door and on all laboratory doors when experiments requiring P3-level containment are in progress. Freezers and refrigerators or other units used to store organisms containing recombinant DNA molecules shall also be posted with the universal biohazard sign.

II-B-3-a-(12). An insect and rodent control program shall be instituted.

II-B-3-a-(13). Laboratory clothing that protects street clothing (e.g., long-sleeve solid-front or wrap-around gowns, no-button or slipover jackets) shall be worn in the laboratory. Front-button laboratory coats are unsuitable. Laboratory clothing shall not be worn outside the laboratory and shall be decontaminated before it is sent to the laundry.

II-B-3-a-(14). Raincoats, overcoats, topcoats, coats, hats, caps, and such street outer-wear shall not be kept in the laboratory.

II-B-3-a-(15). Gloves shall be worn when handling materials requiring P3 containment. They shall be removed aseptically immediately after the handling procedure and decontaminated.

II-B-3-a-(16). Animals and plants not related to the experiment shall not be permitted in the laboratory.

II-B-3-a-(17). Vacuum outlets shall be protected by filter and liquid disinfectant traps.

II-B-3-a-(18). Use of hypodermic needle and syringe shall be avoided when alternative methods are available.

II-B-3-a-(19). The laboratory shall be kept neat and clean.

II-B-3-a-(20). If experiments involving other organisms which require lower levels of containment are to be conducted in the same laboratory concurrently with experiments requiring P3-level physical containment, they shall be conducted in accordance with all P3-level laboratory practices.

II-B-3-b. *Containment Equipment.*

II-B-3-b-(1). Biological safety cabinets [20] shall be used for all equipment and manipulations that produce aerosols—e.g., pipetting, dilutions, transfer operations, plating, flaming, grinding, blending, drying, sonicating, shaking, centrifuging—where these procedures involve organisms containing recombinant DNA molecules, except where equipment design provides for containment of the potential aerosol.

II-B-3-b-(2). Laboratory animals held in a P3 area shall be housed in partial-containment caging systems, such as Horsfall units [19A], open cages placed in ventilated enclosures; solid-wall and -bottom cases covered by filter bonnets, or solid-wall and -bottom cages placed on holding racks equipped with ultraviolet radiation lamps and reflectors.

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

II-B-3-b-(3). *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 in Part III can be conducted in the P3 laboratory using containment equipment specified for the P2 level of physical containment. Experimental procedures involving a host-vector system that provides a one-step lower level of biological containment than that specified in Part III can be conducted in the P3 laboratory using containment equipment specified for the P4 level of physical containment. Alternative combinations of containment safeguards are shown in Table I.

TABLE I.—COMBINATIONS OF CONTAINMENT SAFEGUARDS

Classification of experiment according to guidelines		Alternate combinations of physical and biological containment			
		Physical containment			
Physical containment	Biological[1] containment	Laboratory design specified for—	Laboratory practices specified for—	Containment equipment specified for—	Biological containment
P3	HV3	P3	P3	P3	HV3.
P3	HV3	P3	P3	P4	HV2.
P3	HV2	P3	P3	P3	HV2.
P3	HV2	P3	P3	P2	HV3.
P3	HV2	P3	P3	P4	HV1.
P3	HV1	P3	P3	P3	HV1.
P3	HV1	P3	P3	P2	HV2.

[1] See Section II-D for description of biological containment.

II-B-3-c. *Special Laboratory Design.*

II-B-3-c-(1). The laboratory shall be separated by a controlled access area from areas that are open to unrestricted traffic flow. A controlled access area is an anteroom, a change room, an air lock or any other double-door arrangement that separates the laboratory from areas open to unrestricted traffic flow.

II-B-3-c-(2). The surfaces of walls, floors, and ceilings shall be readily cleanable. Penetrations through these surfaces shall be sealed or capable of being sealed to facilitate space decontamination.

II-B-3-c-(3). A foot-, elbow-, or automatically-operated hand-washing facility shall be provided near each primary laboratory exit area.

II-B-3-c-(4). Windows in the laboratory shall be sealed.

II-B-3-c-(5). An autoclave for sterilization of wastes and contaminated materials shall be available in the same building (and preferably within the controlled laboratory area) in which organisms containing recombinant DNA molecules are used.

II-B-3-c-(6). The laboratory shall have a ventilation system that is capable of controlling air movement. The movement of air shall be from areas of lower contamination potential to areas of higher contamination potential

(i.e., from the controlled access area to the laboratory area). If the ventilation system provides positive pressure supply air, the system shall operate in a manner that prevents the reversal of the direction of air movement or shall be equipped with an alarm that would be actuated in the event that reversal in the direction of air movement were to occur. The exhaust air from the laboratory area shall not be recirculated to other areas of the building unless the exhaust air is filtered by HEPA filters or equivalent. The exhaust air from the laboratory area can be discharged to the outdoors without filtration or other means for effectively reducing an accidental aerosol burden provided that it can be dispersed clear of occupied buildings and air intakes.

II–B–3–c–(7). The treated exhaust-air from Class I and Class II biological safety cabinets [20] may be discharged either to the laboratory or to the outdoors. The treated exhaust-air from a Class III cabinet shall be discharged directly to the outdoors. If the treated exhaust-air from these cabinets is to be discharged to the outdoors through a building exhaust air system, it shall be connected to this system so as to avoid any interference with the air balance of the cabinet and the building ventilation system.

II–B–4. *P4 Level.*

II–B–4–a. *Laboratory Practices.*

II–B–4–a–(1). Laboratory doors shall be kept closed while experiments are in progress.

II–B–4–a–(2). Work surfaces shall be decontaminated following the completion of the experimental activity and immediately following spills of organisms containing recombinant DNA molecules.

II–B–4–a–(3). All laboratory wastes shall be steam-sterilized (autoclaved) before disposal. Other contaminated materials such as glassware, animal cages, laboratory equipment, and radioactive wastes shall be decontaminated by a method demonstrated to be effective before washing, reuse, or disposal.

II–B–4–a–(4). Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

II–B–4–a–(5). Eating, drinking, smoking, and storage of food are not permitted in the P4 facility.

II–B–4–a–(6). Persons shall wash their hands after handling organisms containing recombinant DNA molecules and when they leave the laboratory.

II–B–4–a–(7). Care shall be exercised to minimize the creation of aerosols. For example, manipulations such as inserting a hot inoculating loop or needle into a culture, flaming an inoculation loop or needle so that it splatters, and forceful ejection of fluids from pipettes or syringes shall be avoided.

II–B–4–a–(8). Biological materials to be removed from the P4 facility in a viable or intact state shall be transferred to a nonbreakable sealed container, which is then removed from the P4 facility through a pass-through disinfectant dunk tank or fumigation chamber.

II–B–4–a–(9). No materials, except for biological materials that are to remain in a viable or intact state, shall be removed from the P4 facility unless they have been steam-sterilized (autoclaved) or decontaminated by a means demonstrated to be effective as they pass out of the P4 facility. All wastes and other materials as well as equipment not damaged by high temperature or steam shall be steam sterilized in the double-door autoclave of the P4 facility. Other materials which may be damaged by temperature or steam shall be removed from the P4 facility through a pass-through fumigation chamber.

II–B–4–a–(10). Materials within the Class III cabinets shall be removed from the cabinet system only after being steam-sterilized in an attached double-door autoclave or after being contained in a nonbreakable sealed container, which is then passed through a disinfectant dunk tank or a fumigation chamber.

II–B–4–a–(11). Only persons whose entry into the P4 facility is required to meet program or support needs shall be authorized to enter. Before entering, such persons shall be advised of the nature of the research being conducted and shall be instructed as to the appropriate safeguards to ensure their safety. They shall comply with instructions and all other required procedures.

II–B–4–a–(12). Persons under 18 years of age shall not enter the P4 facility.

II–B–4–a–(13). Personnel shall enter into and exit from the P4 facility only through the clothing change and shower rooms. Personnel shall shower at each egress from the P4 facility. Air locks shall not be used for personnel entry or exit except for emergencies.

II–B–4–a–(14). Street clothing shall be removed in the outer side of the clothing-change area and kept there. Complete laboratory clothing, including undergarments, head cover, shoes, and either pants and shirts or jumpsuits, shall be used by all persons who enter the P4 facility. Upon exit, personnel shall store this clothing in lockers provided for this purpose or discard it into collection hampers before entering the shower area.

II–B–4–a–(15). The universal biohazard sign is required on the P4 facility access doors and on all interior doors to individual laboratory rooms where experiments are conducted. The sign shall also be posted on freezers, refrigerators, or other units used to store organisms containing recombinant DNA molecules.

II–B–4–a–(16). An insect and rodent control program shall be instituted.

II–B–4–a–(17). Animals and plants not related to the experiment shall not be permitted in the laboratory in which the experiment is being conducted.

II–B–4–a–(18). Vacuum outlets shall be protected by filter and liquid disinfectant traps.

II–B–4–a–(19). Use of the hypodermic needle and syringe shall be avoided when alternate methods are available.

II–B–4–a–(20). The laboratory shall be kept neat and clean.

II–B–4–a–(21). If experiments involving other organisms which require lower levels of containment are to be conducted in the P4 facility concurrently with experiments requiring P4-level containment, they shall be conducted in accordance with all P4-level laboratory practices specified in this section.

II–B–4–b. *Containment Equipment.*

II–B–4–b–(1). Experimental procedures involving organisms that require P4-level physical containment shall be conducted either in (i) a Class III cabinet system or in (ii) Class I or Class II cabinets that are located in a specially designed area in which all personnel are required to wear one-piece positive-pressure isolation suits.

II–B–4–b–(2). Laboratory animals in experiments requiring P4-level physical containment shall be housed either in cages contained in Class III cabinets or in partial containment caging systems (such as Horsfall units [19A], open cages placed in ventilated enclosures, or solid-wall and -bottom cages covered by filter bonnets, or solid-wall and -bottom cages placed on holding racks equipped with ultraviolet irradiation lamps and reflectors) that are loated in a specially designed area in which all personnel are required to wear one-piece positive-pressure suits.

II–B–4–b–(3). *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 in Part III can be conducted in the P4 facility using containment equipment requirements specified for

the P3 level of physical containment. Alternative combinations of containment safeguards are shown in Table II.

TABLE II.—COMBINATIONS OF CONTAINMENT SAFEGUARDS

Classification of experiment according to guidelines		Alternate combinations of physical and biological containment			
		Physical containment			
Physical containment	Biological containment [1]	Laboratory design specified for	Laboratory practices specified for	Containment equipment specified for	Biological containment
P4	HV1	P4	P4	P4	HV1.
P4	HV1	P4	P4 [2]	P3	HV2.

[1] See Section II-D for description of biological containment.
[2] In this case gloves shall be worn, in addition to the clothing requirements specified in II-B-4-a-(14).

II-B-4-c. *Special Laboratory Design.*

II-B-4-c-(1). The laboratory shall be located in a restricted-access facility which is either a separate building or a clearly demarcated and isolated zone within a building. Clothing-change areas and shower rooms shall be provided for personnel entry and egress. These rooms shall be arranged so that personnel leave through the shower area to the change room. A double-door ventilated vestibule or ultraviolet air lock shall be provided for passage of materials, supplies, and equipment which are not brought into the P4 facility through the change room area.

II-B-4-c-(2). Walls, floors, and ceilings of the P4 facility are constructed to form an internal shell which readily allows vapor-phase decontamination and is animal- and insect-proof. All penetrations through these structures and surfaces are sealed. (The integrity of the walls, floors, ceilings, and penetration seals should ensure adequate containment of a vapor-phase decontaminant under static pressure conditions. This requirement does not imply that these surfaces must be airtight.)

II-B-4-c-(3). A foot-, elbow-, or automatically-operated handwashing facility shall be provided near the door within each laboratory in which experiments involving recombinant DNA are conducted in openface biological safety cabinets.

II-B-4-c-(4). Central vacuum systems are permitted. The system, if provided, shall not serve areas outside the P4 facility. The vacuum system shall include in-line HEPA filters near each use point or service cock. The filters shall be installed so as to permit in-place decontamination and replacement. Water supply, liquid and gasous services provided to the P4 facility shall be protected by devices that prevent backflow.

II-B-4-c-(5). Drinking water foundations shall not be installed in laboratory or animal rooms of the P4 facilitiay. Foot-operated water fountains are permitted in the corridors of the P4 facility. The water service provided to such fountains shall be protected from the water services to the laboratory areas of the P4 facility.

II-B-4-c-(6). Laboratory doors shall be self-closing.

II-B-4-c-(7). A double-door autoclave shall be provided for sterilization of material passing out of the P4 facility. The autoclave doors shall be interlocked so that both doors will not be open at the same time.

II-B-4-c-(8). A pass-through dunk tank or fumigation chamber shall be provided for removal from the P4 facility of material and equipment that cannot be heat-sterilized.

II-B-4-c-(9). All liquid effluents from the P4 facility shall be collected and decontaminated before disposal. Liquid effluents from biological safety cabinets and laboratory sinks shall be sterilized by heat. Liquid effluents from the shower and hand washing facilities may be activated by chemical treatment. HEPA filters shall be installed in all vents from effluent drains.

II-B-4-c-(10). An individual supply and exhaust-air ventilation system shall be provided. The system shall maintain pressure differentials and directional air flow as required to ensure inflow from areas outside the facility toward areas of highest potential risk within the facility. The system shall be designed to prevent the reversal of air flow. The system shall sound an alarm in the event of system malfunction.

II-B-4-c-(11). Air within individual laboratories of the P4 facility may be recirculated if HEPA filtered.

II-B-4-c-(12). The exhaust air from the P4 facility shall be HEPA filtered and discharged to the outdoors so that it is dispersed clear of occupied buildings and air intakes. The filter chambers shall be designed to allow *in situ* decontamination before removal and to facilitate certification testing after replacement.

II-B-4-c-(13). The treated exhaust-air from Class I and Class II biological safety cabinets [20] may be discharged directly to the laboratory room environment or to the outdoors. The treated exhaust-air from Class III cabinets shall be discharged to the outdoors. If the treated exhaust-air from these cabinets is to be discharged to the outdoors through the P4 facility exhaust air system, it shall be connected to this system so as to avoid any interference with the air balance of the cabinets or the facility exhaust air system.

II-B-4-c-(14). As noted in Section II-B-4-b-(1), the P4 facility may contain specially designed areas in which all personnel are required to wear one-piece positive-pressure isolation suits. Such areas shall be airtight.

The exhaust-air from the suit shall be filtered by two sets of HEPA filters installed in series, and a duplicate filtration unit and exhaust fan shall be provided. The air pressure within the suit area shall be less than that in any adjacent area. An emergency lighting system, communication systems, and power source shall be provided. A double-door autoclave shall be provided for sterilization of all waste materials to be removed from the suit area.

Personnel who enter this area shall wear a one-piece positive-pressure suit that is ventilated by a life-support system. The life-support system shall be provided with alarms and emergency backup air. Entry to this area is through an airlock fitted with airtight doors. A chemical shower area shall be provided to decontaminate the surfaces of the suit before removal.

II-C. *Shipment.* Recombinant DNA molecules contained in an organism or virus shall be shipped only as an etiologic agent under requirements of the U.S. Public Health Service, and the U.S. Department of Transportation § 72.3, Part 72, Title 42, and §§ 173.386-173.388, Part 173, Title 49, U.S. Code of Federal Regulations (CFR) as specified below:

II-C-1. Recombinant DNA molecules contained in an organism or virus requiring P1, P2, or P3 physical containment, when offered for transportation or transported, are subject to all requirements of § 72.3(a)-(e), Part 72, Title 42 CFR, and §§ 173.386-173.388, Part 173, Title 49 CFR.

II-C-2. Recombinant DNA molecules contained in an organism or virus requiring P4 physical containment, when offered for transportation or transported, are subject to the requirements listed above under II-C-1 and are also subject to § 72.3(f), Part 72, Title 42 CFR.

II-C-3. Additional information on packaging and shipment is given in the "Laboratory Safety Monograph—A

Supplement to the NIH Guidelines for Recombinant DNA Research."

II–D. *Biological Containment.*

II–D–1. *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 *H*ost-*V*ector, systems) for prokaryotes will be established; specific criteria will depend on the organisms to be used. Eukaryotic host-vector systems are considered in Part III.

II–D–1–a. *HV1.* A host-vector system which provides a moderate level of containment. *Specific systems:*

II–D–1–a–(1). *EK1.* The host is always *E. coli* K–12 or a derivative thereof, and the vectors include nonconjugative plasmids (e.g., pSC101, ColE1, or derivatives thereof[21–27]) and variants of bacteriophage, such as lambda[28–33]. The *E. coli* K–12 hosts shall not contain conjugation-proficient plasmids, whether autonomous or integrated, or generalized transducing phages, except as specified in Section III–0.

II–D–1–a–(2). *Other Prokaryotes.* Hosts and vectors shall be, at a minimum, comparable in containment to *E. coli* K–12 with a nonconjugative plasmid or bacteriophage vector. The data to be considered and a mechanism for approval of such HV1 systems are described below (Section II–D–2).

II–D–1–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 specified conditions. *Specific systems:*

II–D–1–b–(1). For EK2 host-vector systems in which the vector is a plasmid, no more than one in 10^8 host cells should be able to perpetuate a cloned DNA fragment under the specified nonpermissive laboratory conditions designed to represent the natural environment, either by survival of the original host or as a consequence of transmission of the cloned DNA fragment.

II–D–1–b–(2). For EK2 host-vector systems in which the vector is a phage, no more than one in 10^8 phage particles should be able to perpetuate a cloned DNA fragment under the specified nonpermissible laboratory conditions designed to represent the natural environment either (i) as a prophage (in the inserted or plasmid form) in the laboratory host used for phage propagation or (ii) by surviving in natural environments and transferring a cloned DNA fragment to other hosts (or their resident prophages).

II–D–1–c. *HV3.* These are host-vector systems in which:

II–D–1–c–(1). All HV2 criteria are met.

II–D–1–c–(2). The vector is dependent on its propagation host or is highly defective in mobilizability. Reversion to host-independence must be less than $1/10^8$ per vector genome per generation.

II–D–1–c–(3). No markers conferring resistance to antibiotics commonly used clinically or in agriculture are carried by the vector, unless expression of such markers is dependent on the propagating host or on unique laboratory-controlled conditions or is blocked by the inserted DNA.

II–D–1–c–(4). The specified containment shown by laboratory tests has been independently confirmed by specified tests in animals, including primates, and in other relevant environments.

II–D–1–c–(5). The relevant genotypic and phenotypic traits have been independently confirmed.

II–D–2. *Certification of Host-Vector Systems.*

II–D–2–a. *Responsibility.* HV1 systems other than *E. coli* K–12, and HV2 and HV3 host-vector systems, may not be designated as such until they have been certified by the Director, NIH. Application for certification of a host-vector system is made by written application to the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20205.

Host-vector systems that are proposed for certification will be reviewed by the National Institutes of Health (NIH) Recombinant DNA Advisory Committee (RAC). (See Section IV–E–1–b–(1).) This will first involve review of the data on construction, properties, and testing of the proposed host-vector system by a Working Group composed of one or more members of the RAC and other persons chosen because of their expertise in evaluating such data. The Committee will then evaluate the report of the Working Group and any other available informaton at a regular meeting. The Director, NIH, is responsible for certification after receiving the advice of the RAC. Minor modifications of existing certified host-vector systems, where the modifications are of minimal or no consequence to the properties relevant to containment may be certified by the Director, NIH, without review by the RAC. (See Section IV–E–1–b–(3)–(f).)

When new host-vector systems are certified, notice of the certification will be sent by the Office of Recombinant DNA Activities (ORDA) to the applicant and to all Institutional Biosafety Committees (IBCs) and will be published in the *Recombinant DNA Technical Bulletin.* Copies of a list of all currently certified host-vector systems may be obtained from ORDA at any time.

The Director, NIH, may at any time rescind the certification of any host-vector system. (See Section IV–E–1–b–(3)–(i).) If certification of a host-vector system is rescinded, NIH will instruct investigators to transfer cloned DNA into a different system, or use the clones at a higher physical containment level unless NIH determines that the already constructed clones incorporate adequate biological containment.

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

II–D–2–b. *Data To be Submitted for Certification.*

II–D–2–b–(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.

II–D–2–b–(2). *HV2 Systems.* Investigators planning to request HV2 certification for host-vector systems can obtain instructions from ORDA concerning data to be submitted [33A, 33B]. 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 on transmissibility of the vector and/or a cloned DNA fragment under both permissive and nonpermissive conditions. (v) Data on all other properities 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. (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 (e.g., rodents). Such *in vivo* data may be required to confirm the validity of predicting *in vivo* survival on the basis of *in vitro* experiments.

Data must be submitted in writing to ORDA. Ten to twelve weeks are normally required for review and circulation of the data prior to the meeting at which such data can be considered by the RAC. Investigators are encouraged to publish their data on the construction, properties, and testing of proposed HV2 systems prior to consideration of the system by the RAC and its subcommittee. More specific instructions concerning the type of data to be submitted to NIH for proposed EK2 systems involving either plasmids or bacteriophage in *E. coli* K–12 are available from ORDA.

II–D–2–b–(3). *HV3 Systems.* Putative HV3 systems must, as the first step in certification, be certified as HV2 systems. Systems which meet the critera given above under II–D–1–(c)–1, II–D–1–(c)–2, and II–D–1–(c)–3 will then be recommended for HV3 testing. Tests to evaluate various HV2 host-vector systems for HV3 certification will be performed by contractors selected by NIH. These contractors will repeat tests performed by individuals proposing the HV2 system and, in addition, will conduct more extensive tests on conditions likely to be encountered in nature. The genotypic and phenotypic traits of HV2 systems will be evaluated. Tests on survival and transmissibility in and on animals, including primates, will be performed, as well as tests on survival in certain specified natural environments.

II–D–3. *Distribution of Certified Host-Vectors.* Certified HV2 and HV3 host-vector systems (plus appropriate control strains) must be obtained from the NIH or its designees, one of whom will be the investigator who developed the system. NIH shall announce the availability of the system by publication of notices in appropriate journals.

Plasmid vectors will be provided in a suitable host strain, and phage vectors will be distributed as small-volume lysates. If NIH propagates any of the host strains or phage, a sample will be sent to the investigator who developed the system or to an appropriate contractor, prior to distribution, for verification that the material is free from contamination and unchanged in phenotypic properties.

In distributing the certified HV2 and HV3 host-vector systems, NIH or its designee will (i) send out a complete description of the system; (ii) enumerate and describe the tests to be performed by the user in order to verify important phenotypic traits; (iii) remind the user that any modification of the system necessitates independent approval of the system by the NIH; and (iv) remind the user of responsibility for notifying ORDA of any discrepancies with the reported properties or any problems in the safe use of the system.

NIH may also distribute certified HV1 host-vector systems.

III. Containment Guidelines for Covered Experiments

Part III discusses experiments covered by the Guidelines. The reader must first consult Part I, where listings are given of prohibited and exempt experiments.

Containment guidelines for permissible experiments are given in Part III. For these experiments no registration with the National Institutes of Health (NIH) is necessary. However, for these experiments, prior to their initiation, investigators must submit to their Institutional Biosafety Committee (IBC) a registration document that contains a description of (a) the source(s) of DNA, (b) the nature of the inserted DNA sequences, (c) the hosts and vectors to be used, (d) whether a deliberate attempt will be made to obtain expression of a foreign gene in the cloning vehicle and if so, what protein, and (e) the containment conditions specified by these Guidelines. This registration document must be dated and signed by the investigator and filed only with the local IBC. The IBC shall review all such proposals: IBC review prior to initiation of the experiment is not required for experiments described in Section III–O. Prior IBC review is required for all other experiments described in the subsections of Part III, including III–0–1, III–0–2, etc.

Changes from the levels specified in Part III for specific experiments (or the assignment of levels to experiments not explicitly considered here) may not be instituted without the express approval of the Director, NIH. (See Sections IV–E–1–b–(1)–(a), IV–E–1–b–(1)–(b), IV–E–1–b–(2)–(b), IV–E–1–b–(2)–(c), IV–E–1–b–(3)–(b).)

In the classification of containment criteria for different kinds of recombinant DNAs, the stated levels of physical and biological containment are minimal for the experiments designated. The use of higher levels of biological containment (HV3 > HV2 > HV1) is encouraged if they are available and equally appropriate for the purposes of the experiment.

When the reader finds that the containment level given for the same experiment is different in two different sections within Part III, he may choose whichever of the two levels he wishes to use for the experiment.

III–O. *Classification of Experiments Using Certain Host-Vector Systems.* Experiments listed in Appendix H may be performed at P1 physical containment. For these experiments IBC review prior to initiation of the experiment is not required.

III–O–1. *Experiments Involving Class 3 Organisms.* Experiments involving recombinant DNA from Class 3 organisms [1] or from cells known to be infected with these agents may be conducted at P3 containment in *E. coli* K–12 EK1 hosts (see Appendix C). Containment levels for all other experiments with Class 3 organisms or with recombinant DNA which increases the virulence and host range of a plant pathogen beyond that which occurs by natural genetic exchange will be determined by NIH. (See Section IV–E–1–b–2–(e)).

III–0–2. *Experiments involving Nonpathogenic Prokaryotic and Lower Eukaryotic Host-Vector Systems.* DNA from any species nonpathogenic for man, animals, or plants may be cloned into lower eukaryotes nonpathogenic for man, animals, or plants at the P3 level of

containment [2A]. DNA from any species nonpathogenic for man, animals, or plants may be cloned into prokaryotes nonpathogenic for man, animals, or plants at the P2 level of containment [2A]. Data supporting the contention that the donor and recipient are nonpathogenic must be submitted to the local IBC. Lower levels of physical containment may be assigned by ORDA on a case-by-case basis for specific donor-recipient combinations (see Section IV-E-1-b-(3)-(h)).

III-A. *Classification of Experiments Using Certain HV1 and HV2 Host-Vector Systems.* Certain HV1 and HV2 host-vector systems are assigned containment levels as specified in the subsections of this Section III-A. Those so classified as of publication of these revised Guidelines are listed in Appendix D. An updated list may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20205.

III-A-1. *Shotgun Experiments.* These experiments involve the production of recombinant DNAs between the vector and portions of the specified cellular source, preferably a partially purified fraction. Care should be taken either to preclude or eliminate contaminating microorganisms before isolating the DNA.

III-A-1-a . *Eukaryotic DNA Recombinants.*

III-A-1-a-(1). *Primates.* P2 physical containment + an HV2 host-vector or P3 + HV1.

III-A-1-a-(2). *Other Mammals.* P2 physical containment + an HV2 host-vector or P3 + HV1.

III-A-1-a-(3). *Birds.* P2 physical containment + an HV2 host-vector, or P3 + HV1.

III-A-1-a-(4). *Cold-Blooded Vertebrates.* P2 physical containment + an HV1 host-vector or P1 + HV2. If the eukaryote is known to produce a potent polypeptide toxin, [34] the containment shall be increased to P3 + HV2.

III-A-1-a-(5). *Other Cold-Blooded Animals and Lower Eukaryotes.* This large class of eukaryotes is divided into two groups:

III-A-1-a-(5)-(a). Species that are known to produce a potent polypeptide toxin[34] that acts in vertebrates, or are known pathogens listed in Class 2,[1] or are known to carry such pathogens must use P3 physical containment + an HV2 host-vector. When the potent toxin is not a polypeptide and is likely not to be the product of closely linked eukaryote genes, containment may be reduced to P3 + HV1 or P2 + HV2. Species that produce potent toxins that affect invertebrates or plants but not vertebrates require P2 + HV2 or P3 + HV1. Any species that has a demonstrated capacity for carrying particular pathogenic microorganisms is included in this group, unless the organisms used as the source of DNA have been shown not to contain those agents, in which case they may be placed in the following group.[2A]

III-A-1-a-(5)-(b). The remainder of the species in this class including plant pathogenic or symbiotic fungi that do not produce potent toxins: P2 + HV1 or P1 + HV2. However, any insect in this group must be either (i) grown under laboratory conditions for at least 10 generations prior to its use as a source of DNA, or (ii) if caught in the wild, must be shown to be free of disease-causing microorganisms or must belong to a species that does not carry microorganisms causing disease in vertebrates or plants.[2A] If these conditions cannot be met, experiments must be done under P3 + HV1 or P2 + HV2 containment.

III-A-1-a-(6). *Plants.* P2 physical containment + an HV1 host-vector, or P1 + HV2. If the plant source makes a potent polypeptide toxin,[34] the containment must be raised to P3 physical containment + an HV2 host-vector. When the potent toxin is not a polypeptide and is likely not to be the product of closely linked plant genes, containment may be reduced to P3 + HV1 or P2 + HV2.[2A]

III-A-1-b. *Prokaryotic DNA Recombinants.* P2 + HV1 or P1 + HV2 for experiments with phages, plasmids and DNA from nonpathogenic prokaryotes which do not produce polypeptide toxins.[34] P3 + HV2 for experiments with phages, plasmids and DNA from Class 2 agents.[1]

III-A-2-a. *Viruses of Eukaryotes* (summary given in Table III; see also exception given at asterisk at end of Appendix D).

III-A-2-a-(1). *DNA Viruses.*

III-A-2-a-(1)-(a). *Nontransforming viruses.*

III-A-2-a(1)-(a)-(*1*). *Adeno-Associated Viruses, Minute Virus of Mice, Mouse Adenovirus (Strain FL), and Plant Viruses.[48]* P1 physical containment + and HV1 host-vector shall be used for DNA recombinants produced with (i) the whole viral genome, (ii) subgenomic DNA segments, or (iii) purified cDNA copies of viral mRNA.[37]

III-A-2-a-(1)-(a)-(*2*). *Hepatitis B.*

III-A-2-a-(1)-(a)-(*2*)-(*a*). P1 physical containment + an HV1 host-vector shall be used for purified subgenomic DNA segments.[38]

III-A-2-a-(1)-(a)-(*2*)-(*b*). P2 physical containment + an HV2 host-vector, or P3 + HV1, shall be used for DNA recombinants produced with the whole viral genome or with subgenomic segments that have not been purified to the extent required in footnote 38.

III-A-2-a-(1)-(a)-(*2*)-(*c*). P2 physical containment + an HV1 Host-vector shall be used for DNA recombinants derived from purified cDNA copies of viral mRNA.[37]

III-A-2-a-(1)-(a)-(*3*). *Other Nontransforming Members of Presently Classified Viral Families.[36]*

III-A-2-a-(1)-(a)-(*3*)-(*a*). P1 physical containment + an HV1 host-vector shall be used for (i) DNA recombinants produced with purified subgenomic DNA[38] segments or (ii) purified cDNA copies of viral mRNA.[37]

III-A-2-a-(1)-(a)-(*3*)-(*b*). P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with the whole viral genome or with subgenomic segments that have not been purified to the extent required in footnote 38.

III-A-2-a-(1)-(b). *Transforming Viruses.*[37A]

III-A-2-a-(1)-(b)-(*1*). *Herpes Saimiri, Herpes Ateles, and Epstein Barr Virus.[39]*

III-A-2-a-(1)-(b)-(*1*)-(*a*). P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with purified nontransforming subgenomic DNA segments.[38]

III-A-2-a-(1)-(b)-(*1*)-(*b*). P2 physical containment + an HV1 host-vector shall be used for (i) DNA recombinants produced with purified subgenomic DNA segments containing an entire transforming gene [38] or (ii) purified cDNA copies of viral mRNA.[37]

III-A-2-a-(1)-(b)-(*1*)-(*c*). P3 physical containment + an HV1 host-vector, or P2 + HV2, shall be used for DNA recombinants produced with the whole viral genome or with subgenomic segments that have not been purified to the extent required in footnote 38.

III-A-2-a-(1)-(b)-(*2*). *Other Transforming Members of Presently Classified Viral Families.[36]*

III-A-2-a-(1)-(b)-(*2*)-(*a*). P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with purified nontransforming subgenomic DNA segments.[38]

III-A-2-a-(1)-(b)-(*2*)-(*b*). P2 physical containment + an HV1 host-vector shall be used for (i) DNA recombinants produced with the whole viral genome, (ii) subgenomic DNA segments containing an entire transforming gene, (iii) purified cDNA copies of viral mRNA, [37] or (iv) subgenomic segments

that have not been purified to the extent required in footnote 38.

III–A–2–a–(2). *DNA Transcripts of RNA Viruses.*

III–A–2–a–(2)–(a). *Retroviruses.*

III–A–2–a–(2)–(a)–(*1*). *Gibbon Ape, Woolly Monkey, Feline Leukemia and Feline Sarcoma Viruses. [39]*

III–A–2–a–(2)–(a)–(*1*)–(*a*). P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with purified nontransforming subgenomic DNA segments.[38]

III–A–2–a–(2)–(a)–(*1*)–(*b*). P2 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with purified subgenomic DNA segments[38] containing an entire transforming gene.

III–A–2–a–(2)–(a)–(*1*)–(*c*). P2 physical containment + an HV2 host-vector, or P3 + HV1, shall be used for DNA recombinants produced with (i) the whole viral genome, (ii) purified cDNA copies of viral mRNA,[37] or (iii) subgenomic segments that have not been purified to the extent required in footnote 38.

III–A–2–a–(2)–(a)–(*2*). *Other Members of the Family Retroviridiae.[36]*

III–A–2–a–(2)–(a)–(*2*)–(*a*). P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with purified nontransforming subgenomic DNA segments.[38]

III–A–2–a–(2)–(a)–(*2*)–(*b*). P2 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with (i) subgenomic DNA segments containing an entire transforming gene, (ii) the whole viral genome, or (iii) purified cDNA copies of viral mRNA,[37] or (iv) subgenomic segments that have not been purified to the extent required in footnote 38.

III–A–2–a–(2)–(b). *Negative Strand RNA Viruses.* P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with (i) cDNA copies of the whole genome, (ii) subgenomic cDNA segments, or (iii) purified cDNA copies of viral mRNA.[37]

III–A–2–a–(2)–(c). *Plus-Strand RNA Viruses.*

III–A–2–a–(2)–(c)–(*1*). *Types 1 and 2 Sabin Poliovirus Vaccine Strains and Strain 17D (Theiler) of Yellow Fever Virus.* P1 physical containment + and HV1 host-vector shall be used for DNA recombinants produced with (i) cDNA copies of the whole viral genome, (ii) subgenomic cDNA segments, or (iii) purified cDNA copies of viral mRNA.[37]

III–A–2–a–(2)–(c)–(*2*). *Other Plus-Strand RNA Viruses Belonging to Presently Classified Viral Families.[36]*

III–A–2–a–(2)–(c)–(*2*)–(*a*). P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with purified subgenomic cDNA segments.[38]

TABLE III.—RECOMMENDED CONTAINMENT FOR CLONING OF VIRAL DNA OR cDNA IN CERTAIN HV1 AND HV2 SYSTEMS SPECIFIED IN APPENDIX D

[See text for full details]

Virus class	Type of viral DNA segment to be cloned				
	Subgenomic[38]		Genamic*		cDNA from viral mRNA[37]
	Nontransforming segment	Segment containing an entire transforming gene	Nonsegmented genome	Segmented genome	
DNA:					
Nontransforming viruses:					
AAV, MVM, Mouse Adeno (Strain FL).	P1+HV1		P1+HV1		P1+HV1.
Plant Viruses	P1+HV1		P1+HV1		P1+HV1.
Hepatitis B	P1+HV1[38]		P2+HV2 or P3+HV1		P2+HV1.
Other	P1+HV1[38]		P1+HV1		P1+HV1.
Transforming Viruses:					
Herpes Saimiri, H. Ateles and EBV[39].	P1+HV1[38]	P2+HV1	P2+HV2 or P3+HV1		P2+HV1.
Other	P1+HV1[38]	P2+HV1	P2+HV1		P2+HV1*.
RNA:					
Retroviruses:					
Gibbon Ape, Woolly Monkey FeLV and FeSV[39].	P1+HV1[38]	P2+HV1	P2+HV2 or P3+HV1		P2+HV2 or P3+HV1.
Other	P1+HV1[38]	P2+HV1	P2+HV1		P2+HV1.
Negative-Strand RNA	P1+HV1		P1+HV1	P1+HV1	P1+HV1.
Plus-Strand RNA:					
Types 1 and 2 Sabin Polio, 17D Yellow Fever Vaccine Strains.	P1+HV1		P1+HV1		P1+HV1.
Other	P1+HV1[38]		P2+HV1		P2+HV1.
Double-Stranded RNA	P1+HV1			P1+HV1	P1+HV1.
Plant Viruses + Viroids	P1+HV1		P1+HV1	P1+HV1	P1+HV1.
Intracellular Viral DNA	See text	See text	See text		

*See exception given at asterisk at end of Appendix D.

III–A–2–a–(2)–(c)–(*2*)–(*b*). P2 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with (i) cDNA copies of the whole genome, or (ii) purified cDNA copies of viral mRNA.[37]

III–A–2–a–(2)–(d). *Double-Stranded Segmented RNA Viruses.* P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with (i) mixtures of subgenomic cDNA segments, (ii) a specific subgenomic cDNA segment, or (iii) purified cDNA copies of viral mRNA.[37]

III–A–2–a–(2)–(e). *RNA Plant Viruses and Plant Viroids.*[48] P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with (i) cDNA copies of the whole viral genome, (ii) subgenomic cDNA segments, or (iii) purified cDNA copies of viral mRNA.[37]

III–A–2–a–(3). *Intracellular Viral DNA.* Physical and biological containment specified for shotgun experiments with eukaryotic cellular DNA [see Section III–A–(1)–(a)] shall be used for DNA recombinants produced with integrated viral DNA or viral genomes present in infected cells.

III–A–2–b. *Eukaryotic Organelle DNAs.* P2 physical containment + an HV1 host-vector, or P1 + HV2, for mitochondrial or chloroplast DNA from eukaryotes when the organelle DNA has been obtained from isolated organelles. Otherwise, the conditions given for shotgun experiments apply.

III–A–2–c. *Prokaryotic Plasmid and Phage DNAs.* The containment levels required for shotgun experiments with DNA from prokaryotes apply to their

plasmids or phages (See Section III–A–1–b.)

III–A–3. *Lowering of Containment Levels for Characterized or Purified DNA Preparations and Clones.* Many of the risks which might conceivably arise from some types of recombinant DNA experiments, particularly shotgun experiments, would result from the inadvertent cloning of a harmful sequence. Therefore, in cases where the risk of inadvertently cloning the "wrong" DNA is reduced by prior enrichment for the desired piece, or in which a clone made from a random assortment of DNAs has been purified and the absence of harmful sequences established, the containment conditions for further work may be reduced. The following section outlines the mechanisms for such reductions.

III–A–3–a. *Purified DNA Other than Plasmids, Bacteriophages, and Other Viruses.* The formation of DNA recombinants from cellular DNAs that have been purified[41] and in which the absence of harmful sequences has been established[3] can be carried out under lower containment conditions than used for the corresponding shotgun experiment.[42] The containment may be decreased one step in physical containment (P4 P3; P3 P2; P2 P1) while maintaining the biological containment specified for the shotgun experiment, or one step in biological containment (HV3 HV2; HV2 HV1) while maintaining the specified physical containment. The Institutional Biosafety Committee (IBC) must review such a reduction and the approval of the IBC and of the NIH must be secured before such a reduction may be put into effect. IBC approval is sufficient for such a reduction except for any lowering of containment under Section III–A–3–a to levels below P1+HV1, which requires prior NIH approval. (See Section IV–E–1–b–(3)–(e).)

III–A–3–b. *Characterized Clones of DNA Recombinants.* When a cloned DNA recombinant has been rigorously characterized and the absence of harmful sequences has been established[3], experiments involving this recombinant DNA may be carried out under lower containment conditions. Institutional Biosafety Committees (IBCs) may give approval for a single-step reduction in physical or biological containment on receipt of evidence of characterization of a clone derived from a shotgun experiment and its probable freedom from harmful genes. IBC approval is sufficient for such a reduction except for any lowering of containment under Section III–A–3–b to levels below P1+HV1, or reduction of containment levels by more than one step, which also requires prior NIH approval.(See Section IV–E–1–b–3–(e).)

III–B. *Experiments with Prokaryotic Host-Vectors Other Than E. coli K–12.*

III–B–1. *HV1 and HV2 Systems.* Certain certified HV1 and HV2 hostvector systems appear in Appendix D. The containment levels for these systems are given in the subsections of Section III–A. Other systems in the future may be certified as HV1 and HV2. At the time of certification, the classification of containment levels for experiments using them will be assigned by NIH.

III–B–2. *Return of DNA Segments to Prokaryotic Non–HV1 Host of Origin.* Certain experiments involving these prokaryotes that exchange genetic information with *E. coli* by known physiological processes will be exempt from these Guidelines if they appear on the "list of exchangers" set forth in Appendix A (see Section I–E–4). For a prokaryote which can exchange genetic information[35] with *E. coli* under laboratory conditions but which is not on the list (Host A), the following type of experiment may be carried out under P1 conditions without Host A having been approved as an HV1 host: DNA from Host A may be inserted into a vector and propagated in *E. coli* K–12. Subsequently, this recombinant DNA may be returned to Host A by mobilization, transformation, or transduction and may then be propagated in Host A in any desired vector under P1 conditions.

For a prokaryote which does not exchange genetic information with *E. coli* (Host B), the following type of experiment may be carried out without Host B having been approved as an HV1 host: DNA from Host B may be inserted into a vector and propagated in *E. coli* K–12. Subsequently, this recombinant DNA may be returned to Host B and propagated in Host B under P1 conditions.[43]

III–B–3. *Non-HV1 Systems.* Containment levels for other classes of experiments involving non-HV1 systems may be approved by the Director, NIH. (See Sections IV–E–1–b–(1)–(b), IV–E–1–b–(2)–(c), and IV–E–1–b–(3)–(b).)

III–C. *Experiments with Eukaryotic Host-Vectors.*

III–C–1. *Vertebrate Host-Vector Systems.[44]* The subsections of Sections III–C–1–a, –b, –c and –d involve the use of specific viral vectors, namely polyoma, SV40, human adenoviruses 2 and 5, and mouse adenovirus strain FL, respectively. The subsections of Section III–C–1–e involve the use of viral vectors including the specific viral vectors considered in the subsections of Sections III–C–1–a, –b, –c and –d, as well as any other viral vector. When the reader finds that the containment level given for a specific experiment in a subsection of Section III–C–1–e is different from the containment level given in a subsection of Section III–C–1–a, –b, –c or –d, he may choose which of the two containment levels he wishes to use for the experiment.

III–C–1–a. *Polyoma Virus.*

III–C–1–a–(1). *Productive Virus-Cell Interactions.*

III–C–1–a–(1)–(a). Defective or whole polyoma virus genomes, with appropriate helper, if necessary, can be used in P2 conditions to propagate DNA sequences:

III–C–1–a–(1)–(a)–(*1*). from bacteria of Class 1 or Class 2[1] or their phages or plasmids, except for those that produce potent polypeptide toxins;[34]

III–C–1–a–(1)–(a)–(*2*). from mice;

III–C–1–a–(1)–(a)–(*3*). from eukaryotic organisms that do not produce potent polypeptide toxins,[34] provided that the DNA segment is ⩾99% pure.

III–C–1–a–(1)–(b). Defective polyoma genomes, with appropriate helper, if necessary, can be used in P2 conditions for shotgun experiments to propagate DNA sequences from eukaryotic organisms that do not produce potent polypeptide toxins.[34]

III–C–1–a–(1)–(c). Whole virus genomes with appropriate helper, if necessary, can be used in P3 conditions for shotgun experiments to propagate DNA sequences from eukaryotic organisms that do not produce potent polypeptide toxins.[34]

III–C–1–a–(1)–(d). Experiments involving the use of defective polyoma virus genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by NIH on a case-by-case basis[45] and will be conducted under the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

III–C–1–a–(2). *Nonproductive Virus-Cell Interactions.* Defective or whole polyoma virus genomes can be used as vectors in P2 conditions when production of viral particles cannot occur (e.g., transformation of nonpermissive cells or propagation of an unconditionally defective recombinant genome in the absence of helper): *Provided,* The inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis[45] and will be conducted under the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

III-C-1-b. *Simian Virus 40.*

III-C-1-b-(1). *Productive Virus-Cell Interactions.*

III-C-1-b-(1)-(a). SV40 DNA, rendered unconditionally defective by a deletion in an essential gene, with appropriate helper, can be used in P2 conditions to propagate DNA sequences from:

III-C-1-b-(1)-(a)-(*1*). bacteria of Class 1 or Class 2,[1] or their phages or plasmids, except for those that produce potent polypeptide toxins;[34]

III-C-1-b-(1)-(a)-(*2*). uninfected African green monkey kidney cell cultures.

III-C-1-b-(1)-(b). SV40 DNA, rendered unconditionally defective by a deletion in an essential gene, with an appropriate helper, can be used in P3 conditions to propagate DNA sequences from eukaryotic organisms that do not produce potent polypeptide toxins[34] (shotgun experiments or purified DNA).

III-C-1-b-(1)-(c). Experiments involving the use of defective SV40 genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by NIH on a case-by-case basis[45] and will be conducted under the prescribed physical and biological containment conditions. (See Section IV-E-1-b-(3)-(c).)

III-C-1-b-(2). *Nonproductive Virus-Cell Interactions.* Defective or whole SV40 genomes can be used as vectors in P2 conditions when production of viral particles cannot occur (e.g., transformation of nonpermissive cells or propagation of an unconditionally defective recombinant genome in the absence of helper): *Provided,* The inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis[45] and will be conducted under the prescribed physical and biological containment conditions. (See Section IV-E-1-b-(3)-(c).)

III-C-1-c. *Human Adenoviruses 2 and 5.*

III-C-1-c-(1). *Productive Virus-Cell Interactions.*

III-C-1-c-(1)-(a). Human adenoviruses 2 and 5, rendered unconditionally defective by deletion of at least two essential genes, with appropriate helper, can be used in P3 conditions to propagate DNA sequences from:

III-C-1-c-(1)-(a)-(*1*). bacteria of Class 1 or Class 2[1] or their phages or plasmids except for those that produce potent polypeptide toxins;[34]

III-C-1-c-(1)-(a)-(*2*). eukaryotic organisms that do not produce potent polypeptide toxins[34] (shotgun experiments or purified DNA).

III-C-1-c-(1)-(b). Experiments involving the use of unconditionally defective human adenovirus 2 and 5 genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by NIH on a case-by-case basis[45] and will be conducted under the prescribed physical and biological containment conditions. (See Section IV-E-1-b-(3)-(c).)

III-C-1-c-(2). *Nonproductive Virus-Cell Interactions.* Defective or whole human adenovirus 2 and 5 genomes can be used as vectors in P2 conditions when production of viral particles cannot occur (e.g., transformation of nonpermissive cells or propagation of an unconditionally defective recombinant genome in the absence of helper); *Provided,* The inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis[45] and will be conducted under the prescribed physical and biological containment conditions. (See Section IV-E-1-b-(3)-(c).)

III-C-1-d. *Murine Adenovirus Strain FL.*

III-C-1-d-(1). *Productive Virus-Cell Interactions.*

III-C-1-d-(1)-(a). Unconditionally defective murine adenovirus strain FL genomes, with appropriate helper, can be used in P2 conditions to propagate DNA sequences from:

III-C-1-d-(1)-(a)-(*1*). bacteria of Class 1 or Class 2[1] or their phages or plasmids except for those that produce potent polypeptide toxins;[34]

III-C-1-d-(1)-(a)-(*2*). eukaryotic organisms that do not produce potent polypeptide toxins[34] (shotgun experiments or purified DNA).

III-C-1-d-(1)-(b). Experiments involving the use of whole murine adenovirus strain FL genomes to propagate DNA sequences from prokaryotic or eukaryotic organisms will be evaluated by NIH on a case-by-case basis[45] and will be conducted under the prescribed physical and biological containment conditions. (See Section IV-E-1-b-(3)-(c).)

III-C-1-d-(1)-(c). Experiments involving the use of unconditionally defective murine adenovirus strain FL genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by NIH on a case-by-case basis[45] and will be conducted under the prescribed physical and biological containment conditions. (See Section IV-E-1-b-(3)-(c).)

III-C-1-d-(2). *Nonproductive Virus-Cell Interactions.* Defective or whole murine adenovirus strain FL genomes can be used as vectors in P2 conditions when production of viral particles cannot occur (e.g., transformation of nonpermissive cells or propagation of an unconditionally defective recombinant genome in the absence of helper); *Provided,* The inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis [45] and will be conducted under the prescribed physical and biological containment conditions. (See Section IV-E-1-b-(3)-(c).)

III-C-1-e. *All Viral Vectors.*

III-C-1-e-(1). Other experiments involving eukaryotic virus vectors can be done as follows:

III-C-1-(1)-(a). Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus [all viruses from a single Family (36) being considered identical (50)] may be propagated and maintained in cells in tissue culture using P1 containment. For such experiments, it must be shown that the cells lack helper virus for the specific Families of defective viruses being used. The DNA may contain fragments of the genomes of viruses from more than one Family but each fragment must be less than two-thirds of a genome.

III-C-1-e-(1)-(b). Recombinants with less than two-thirds of the genome of any eukaryotic virus may be rescued with helper virus using P2 containment if wild type strains of the virus are CDC Class 1 or 2 agents, or using P3 containment if wild type strains of the virus are CDC Class 3 agents (1).

III-C-1-e-(2). Experiments involving the use of other whole or defective virus genomes to propagate DNA sequences from prokaryotic or eukaryotic organisms (and viruses), or as vectors to transform nonpermissive cells, will be evaluated by NIH on a case-by-case basis [45] and will be conducted under the prescribed physical and biological containment conditions. (See Section IV-E-1-b-(3)-(c).)

NIH will also review on a case-by-case basis[45] all experiments involving the use of virus vectors in animals and will prescribed the physical and biological containment conditions appropriate for such studies. (See Section IV-E-1-b-(3)-(c).)

III-C-1-f. *Nonviral Vectors.* Organelle, plasmid, and chromosomal DNAs may be used as vectors. DNA recombinants formed between such vectors and host DNA, when propagated only in that host (or a closely related strain of the same species), are exempted from these Guidelines (see Section I-E). DNA recombinants formed between such vectors and nonviral DNA from cells other than the host species

require only P1 physical containment for cells in culture since vertebrate cells in tissue culture inherently exhibit a very high level of containment. Recombinants involving viral DNA or experiments which require the use of the whole animals will be evaluated by NIH on a case-by-case basis.[45]

III–C–2. *Invertebrate Host-Vector Systems.*

III–C–2–a. *Inverebrate Viral Vectors.* Experiments involving invertebrate virus vectors can be done as follows:

III–C–2–a–(1). Recombinant DNA molecules containing no more than two-thirds of the genome of any invertebrate virus (all viruses from a single Family (36) being considered identical (50)) may be propagated and maintained in cells in tissue culture using P1 containment. For such experiments, it must be shown that the cells lack helper virus for the specific Families of defective viruses being used. The DNA may contain fragments of the genomes of viruses from more one Family but each fragment must be less than two-thirds of a genome.

III–C–2–a–(2). Recombinants with less than two-thirds of the genome of any invertebrate virus may be rescued with helper virus using P2 containment unless it is classified by the CDC as a class 3 agent (1) in which case P3 containment is required.

III–C–2–a–(3). Experiments involving the use of other whole or defective virus genomes to propagate DNA sequences from prokaryotic or eukaryotic organisms (and viruses), or as vectors to transform nonpermissive cells, will be evaluated by NIH on a case-by-case basis [45] and will be conducted under the prescribed physical and biological containment condtions. (See Section IV–E–1–b–(3)–(c).)

NIH will also review on a case-by-case basis [45] all experiments involving the use of virus vectors in animals and will prescribe the physical and biological containment conditions appropriate for such studies. (See Section IV–E–1–b–(3)–(c).)

III–C–2–b. *Nonviral Vectors.* Organelle, plasmid, and chromosomal DNAs may be used as vectors. DNA recombinants formed between such vectors and host DNA, when propagated only in that the host (or a closely related strain of the same species), are exempt from these Guidelines (see Section I–E). DNA recombinants formed between such vectors and DNA from cells other than the host species require P1 physical containment for invertebrate cells in culture inherently exhibits a very high level of containment. Experiments which require the use of whole animals will be evaluated by NIH on a case-by-case basis.[45]

III–C–3. *Plant Viral Host-Vector Systems.* [48] The DNA plant viruses which could currently serve as vectors for coloning genes in plants and plant cell protoplasts are Cauliflower Mosaic Virus (CaMV) and its close relatives [2A] which have relaxed circular double-stranded DNA genomes with a molecular weight of 4.5×10^6, and Bean Golden Mosaic Virus (BGMV) and related viruses with small ($>10^6$ daltons) single-stranded DNA genomes. CaMV is spread in nature by aphids, in which it survives for a few hours. Spontaneous mutants of CaMV which lack a factor essential for aphid transmission arise frequently. BGMV is spread in nature by whiteflies, and certain other single-stranded DNA plant viruses are transmitted by leafhoppers.

The DNA plant viruses have narrow host ranges and are relatively difficulty to transmit mechanically to plants. For this reason, they are most unlikely to be accidentally transmitted from spillage of purified virus preparations.

When these viruses are used as vectors in intact plants, or propagative plant parts, the plants shall be grown under P1 conditions—that is, in either a limited access greenhouse or plant growth cabinet which is insect-restrictive, preferably with positive air pressure, [2A] and in which an insect fumigation regime is maintained. Soil, plant pots, and unwanted infected materials shall be removed from the greenhouse or cabinet in sealed insect-proof containers and sterilized. It is not necessary to sterilize run-off water from the infected plants, as this is not a plausible route for secondary infection. When the viruses are used as vectors in tissue cultures or in small plants in axenic cultures, no special containment is necessary. Infected plant materials which have to be removed from the greenhouse or cabinet for further research shall be maintained under insect-restrictive conditions. These measures provide an entirely adequate degree of containment. They are similar to those required in many countries for licensed handling of "exotic" plant viruses.

The viruses or their DNA may also be useful as vectors to introduce genes into plant protoplasts. The fragility of plant protoplasts combined with the properties of the viruses provides adequate safety. Since no risk to the environment from the use of the DNA plant virus/protoplast system is envisaged, no special containment is necessary, except as described in the following paragraph.

Experiments involving the use of plant genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by NIH on a case-by-case basis [45] and will be conducted under the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

III–C–4. *Plant Host-Vector Systems Other than Viruses.* [48] Organelle, plasmid, and chromosomal DNAs may be used as vectors. DNA recombinants formed between such vectors and host DNA, when propagated only in that host (or a closely related strain of the same species), are exempt from these Guidelines (see Section I–E). DNA recombinants formed between such vectors and DNA from cells other than the host species require P2 physical containment. The development of host-vector systems that exhibit a high level of biological containment, such as those using protoplasts or undifferentiated cells in culture, permit [2A] a decrease in the physical containment to P1.

Intact plants or propagative plant parts which cannot be grown in a standard P2 laboratory because of their large size may be grown under the P1 conditions described above in Section III–C–3, except that (i) sterilization of run-off water is required where this is a plausible route for secondary infection and (ii) the standard P2 practices are adopted for microbiological work, and (iii) negative air pressure should be employed in the greenhouse or growth chamber when infectious agents are used which generate airborne propagules.

III–C–5. *Fungal or Similar Lower Eukaryotic Host-Vector Systems.*

Certain certified HV1 and HV2 host-vector systems appear in Appendix D. The containment levels for these systems are given in the subsections of Section III–A. Other systems in the future may be certified as HV1 and HV2. At the time of certification, they may be added to Appendix D (and thus the containment levels for their use will be those of the subsections of Section III–A). Alternatively, at the time of their certification, another classification of containment levels for experiments using them may be assigned by NIH.

In addition to the experiments described above, the following experiments may be carried out without the eukaryotic host (Host C) having been approved as an HV1 host: DNA from Host C may be inserted into a vector and propagated in *E. coli* K–12. Subsequently, this recombinant DNA may be returned to Host C and propagated there under P1 conditions.[43]

Containment levels for other classes of experiments involving non-HV1 systems may be expressly approved by the Director, NIH. (See Sections IV-E-1-b-(1)-(b), IV-E-1-b-(2)-(c), and IV-E-1-b-(3)-(b).)

III-C-6. *Return of DNA Segments to a Higher Eukaryotic Host of Origin.* DNA from a higher eukaryote (Host D) may be inserted into a vector and propagated in *E. coli* K-12. Subsequently, this recombinant DNA may be returned to Host D and propagated under conditions of physical containment comparable to P1 and appropriate to the organism under study.[2A]

III-C-7. *Transfer of Cloned DNA Segments to Eukaryotic Organisms.*

III-C-7-a. *Transfer to Non-human Vertebrates.* DNA from any nonprohibited source (Section I-D), except for greater than one quarter of a eukaryotic viral genome, which has been cloned and propagated in *E. coli* K-12, may be transferred with *E. coli* vector used for cloning to any eukaryotic cells in culture or to any non-human vertebrate organism and propagated under conditions of physical containment comparable to P1 and appropriate to the organism under study[2]. Transfers to any other host will be considered by the RAC on a case-by-case basis[45].

III-C-7-b. *Transfer to Higher Plants.* DNA from any nonprohibited source [Section I-D] which has been cloned and propagated in *E. coli* K-12 or *S. cerevisiae,* may be transferred with the *E. coli* or *S. cerevisiae* vector used for cloning to any higher plant organisms (Angiosperms and Gymnosperms) and propagated under conditions of physical containment comparable to P1 and appropriate to the organism under study [2A]. Intact plants or propagative plant parts may be grown under P1 conditions described under Section III-C-3. Containment must be modified to ensure that the spread of pollen, seed or other propagules is prevented. This can be accomplished by conversion to negative pressure in the growth cabinet or greenhouse or by physical entrapment by "bagging" of reproductive structures. Transfers to any other plant organisms will be considered on a case-by-case basis[45].

III-C-7-c. *Transfer to Invertebrates.* DNA from any nonprohibited source [Section I-D], except for greater than one quarter of a eukaryotic viral genome, which has been cloned and propagated in *E. coli* K-12, may be transferred with the *E. coli* vector used for cloning to any eukaryotic cells in culture or to any invertebrate organism and propagated under conditions of physical containment comparable to P1 and appropriate to the organism under study (2A). Transfers to any other host will be considered by the RAC on a case-by-case basis (45).

III-D. *Complementary DNAs.* Specific containment levels are given in Section III-A-2-a (see also last column of Table III) for complementary DNA (cDNA) of viral mRNA. For the other Sections of the Guidelines, where applicable, cDNAs synthesized *in vitro* are included within each of the above classifications. For example, cDNAs formed from cellular RNAs that are not purified and characterized are included under III-A-1, shotgun experiments; cDNAs formed from purified and characterized RNAs are included under III-A-3; etc.

Due to the possibility of nucleic acid contamination of enzyme preparations used in the preparation of cDNAs, the investigator must employ purified enzyme preparations that are free of viral nucleic acid.

III-E. *Synthetic DNAs.* If the synthetic DNA segment is likely to[2A] yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent), the containment conditions must be as stringent as would be used for propagating the natural DNA counterpart.

If the synthetic DNA sequence codes for a harmless product,[2A] it may be propagated at the same containment level as its purified natural DNA counterpart. For example, a synthetic DNA segment which corresponds to a nonharmful gene of birds, to be propagated in *Saccharomyces cerevisiae,* would require P2 physical containment plus an HV1 host-vector, or P1 + HV2.

If the synthetic DNA segment is not expressed *in vivo* as a polynucleotide or polypeptide product, the organisms containing the recombinant DNA molecule are exempt[4] from the Guidelines.

IV. Roles and Responsibilities

IV-A. *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, the Institutional Biosafety Committee (IBC), the Biological Safety Officer, and the Principal Investigator 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 *purpose* of the Guidelines as well as to their 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. Further clarifications and interpretations of roles and responsibilities will be issued by NIH as necessary.

IV-B. *General Applicability.* The Guidelines are applicable to all recombinant DNA research within the United States or its territories which is conducted at or sponsored by an Institution that receives any support for recombinant DNA research from NIH. This includes research by NIH directly.

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.

The Guidelines are also applicable to projects done abroad if they are supported by NIH funds. If the host country, however, has established rules for the conduct of recombinant DNA projects, then a certificate of compliance with those rules may be submitted to NIH in lieu of compliance with the NIH Guidelines. NIH reserves the right to withhold funding if the safety practices to be employed abroad are not reasonably consistent with the NIH Guidelines.

IV-C. *General Definitions.* The following terms, which are used throughout the Guidelines, are defined as follows:

IV-C-1. "DNA" means deoxyribonucleic acid.

IV-C-2. "Recombinant DNA" or "recombinant DNA molecules" means either (i) 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 which result from the replication of a molecule described in (i) above.

IV-C-3. [Deleted]

IV-C-4. "Institution" means any public or private entity (including

Federal, State, and local government agencies).

IV-C-5. "Institutional Biosafety Committee" of "IBC" means a committee that (i) meets the requirements for membership specified in Section IV-D-2, and (ii) reviews, approves, and oversees projects in accordance with the responsibilities defined in Sections IV-D-2 and -3.

IV-C-6. "NIH Office of Recombinant DNA Activities" or "ORDA" means the office within NIH with responsibility for (i) reviewing and coordinating all activities of NIH related to the Guidelines, and (ii) performing other duties as defined in Section IV-E-3.

IV-C-7. "Recombinant DNA Advisory Committee" or "RAC" means the public advisory committee that advises the Secretary, the Assistant Secretary for Health, and the Director of the National Institutes of Health concerning recombinant DNA research. The RAC shall be constituted as specific in Section IV-E-2.

IV-C-8. "Director, NIH" or "Director" means the Director of the National Institutes of Health and any other officer or employee of NIH to whom authority has been delegated.

IV-C-9. "Federal Interagency Advisory Committee on Recombinant DNA Research" means the committee established in October 1976 to advise the Secretary, HHS, the Assistant Secretary of Health, and the Director, NIH, on the coordination of those aspects of all Federal programs and activities which relate to recombinant DNA research.

IV-C-10. "Administrative Practices Supplement" or "APS" means a publication to accompany the NIH Guidelines specifying administrative procedures for use at NIH and at Institutions.

IV-C-11. "Laboratory Safety Monograph" or "LSM" means a publication to accompany the NIH Guidelines describing practices, equipment, and facilities in detail.

IV-D. *Responsibilities of the Institution.*

IV-D-1. Each Institution conducting or sponsoring recombinant DNA research covered by these Guidelines is responsible for ensuring that the research is carried out in full conformity with the provisions of the Guidelines. In order to fulfill this responsibility, the Institution shall:

IV-D-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.

IV-D-1-b. Establish an Institutional Biosafety Committee (IBC) that meets the requirements set forth in Section IV-D-2 and carries out the functions detailed in Section IV-D-3.

IV-D-1-c. [Deleted]

IV-D-1-d. [Deleted]

IV-D-1-e. If the Institution is engaged in recombinant DNA research at the P3 or P4 containment level, appoint a Biological Safety Officer (BSO), who shall be a member of the IBC and carry out the duties specified in Section IV-D-4.

IV-D-1-f. Require that investigators responsible for research covered by these Guidelines comply with the provisions of Section IV-D-5, and assist investigators to do so.

IV-D-1-g. Ensure appropriate training for the IBC chairperson and members, the BSO, Principal Investigators (PIs), 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.

IV-D-1-h. 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) 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.)

IV-D-1-i. Report within 30 days to ORDA any significant problems with and violations of the Guidelines and significant research-related accidents and illnesses, unless the institution determines that the PI or IBC has done so.

IV-D-2. *Membership and Procedures of the IBC.* The Institution shall establish an Institutional Biosafety Committee (IBC) meeting the following requirements:

IV-D-2-a. 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 (but not less than 20 percent of the membership of the committee) 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 the 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 Biological Safety Officer (BSO), mandatory when research is being conducted at the P3 and P4 levels, shall be a member (see Section IV-D-4).

IV-D-2-b. In order to ensure the professional competence necessary to review recombinant DNA activities, it is recommended that (i) the IBC include persons from disciplines relevant to recombinant DNA technology, biological safety, and engineering; (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 a nondoctoral person from a laboratory technical staff.

IV-D-2-c. The Institution shall identify the committee members by name in a report to the NIH Office of Recombinant DNA Activities (ORDA) and shall include relevant background information on each member in such form and at such times as ORDA may require. (See the Administrative Practices Supplement for further guidance.)

IV-D-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.

IV-D-2-e. The Institution 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 IV-D-3-a.)

IV-D-2-f. Central to implementation of the Guidelines is the review of experiments by the IBC. In carrying out this responsibility, the Institution shall comply with instructions and procedures specified in the Administrative Practices Supplement.

IV-D-2-g. Institutions are encouraged to open IBC meetings to the public whenever possible, consistent with protection or privacy and proprietary interests.

IV-D-2-h. Upon request, the Institution shall made 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 (e.g., reports of Guideline violations and significant research-related accidents, and agency directives to modify projects). If comments are made by members of the public on IBC actions, the Institution shall forward to NIH both the comments and the IBC's response.

IV-D-3. *Functions of the IBC.* On behalf of the Institution, the IBC is responsible for:

IV-D-3-a. Reviewing for compliance with the NIH Guidelines all recombinant DNA research 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:

IV-D-3-a-(1). An independent assessment of the containment levels required by these Guidelines for the proposed research, and

IV-D-3-a-(2). An assessment of the facilities, procedures, and practices, and of the training and expertise of recombinant DNA personnel.

Note.—See Laboratory Safety Monograph (pages 187–190) for suggested guidance in conducting this review.

IV-D-3-b. Notifying the Principal Investigator (PI) of the results of their review.

IV-D-3-c. Reviewing periodically recombinant DNA research being conducted at the Institution, to ensure that the requirements of the Guidelines are being fulfilled.

IV-D-3-d. Adopting emergency plans covering accidental spills and personnel contamination resulting from such research.

Note.—Basic elements in developing specific procedures for dealing with major spills of potentially hazardous materials in the laboratory are detailed in the Laboratory Safety Monograph. Included are information and references on decontamination and emergency plans. NIH and the Centers for Disease Control are available to provide consultation, and direct assistance if necessary, as posted in the LSM. The Institution shall cooperate with the State and local public health departments, reporting any significant research-related illness or accident that appears to be a hazard to the public health.

IV-D-3-e. Reporting within 30 days to the appropriate institutional official and to the NIH Office of Recombinant DNA Activities (ORDA) any significant problems with or violations of the Guidelines, and any significant research-related accidents or illnesses, unless the IBC determines that the PI has done so.

IV-D-3-f. The IBC may not authorize initiation of experiments not explicitly covered by the Guidelines until NIH, (with the advice of the RAC when required) established the containment requirement.

IV-D-3-g. Performing such other functions as may be delegated to the IBC under Section IV-D-1.

IV-D-4. *Biological Safety Officer.* The Institution shall appoint a BSO if it engages in recombinant DNA research at the P3 or P4 containment level. The officer shall be a member of the Institutional Biosafety Committee (IBC), and his or her duties shall include (but need not be limited to):

IV-D-4-a. Ensuring through periodic inspections that laboratory standards are rigorously followed;

IV-D-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 (PI) has done so.

IV-D-4-c. Developing emergency plans fro dealing with accidential spills and personnel contamination, and investigating recombinant DNA research laboratory accidents;

IV-D-4-d. Providing advice on laboratory security;

IV-D-4-e. Providing technical advice to the PI and IBC on research safety procedures.

Note.—See Laboratory Safety Monograph for additional information on the duties of the BSO.

IV-D-5. *Principal Investigator.* On behalf of the Institution, the PI is responsible for complying fully with the Guidelines in conducting any recombinant DNA research.

IV-D-5-a. *PI-General.* As part of this general responsibility, the PI shall:

IV-D-5-a-(1). Initiate or modify no recombinant DNA research subject to the Guidelines until that research, or the proposed modification thereof, has been approved by the Institutional Biosafety Committee (IBC) and has met all other requirements of the Guidelines and the Administrative Practices Supplement (APS).

Note.—No prior approval by the IBC is required for most experiments described in Section III-O. Modify containment and experimental protocol according to recommendations of the IBC.

IV-D-5-a-(2). Report within 30 days to the IBC and NIH (ORDA) all significant problems with and violations of the Guidelines and all significant research-related accidents and illnesses;

IV-D-5-a-(3). Report to the IBC and to NIH (ORDA) new information bearing on the Guidelines;

IV-D-5-a-(4). Be adequately trained in good microbiological techniques;

IV-D-5-a-(5). Adhere to IBC-approved emergency plans for dealing with accidental spills and personnel contamination; and

IV-D-5-a-(6). Comply with shipping requirements for recombinant DNA molecules. (See Section II-C for shipping requirements, Laboratory Safety Monograph for technical recommendations, and the APS for administrative instructions and procedures. The requesting laboratory must be in compliance with the NIH Guidelines and under appropriate review by its IBC, and the sending investigator must maintain a record of all shipments of recombinant DNA materials.)

IV-D-5-b. *Submissions by the PI to NIH.* The PI shall:

IV-D-5-b-(1). Submit information to NIH (ORDA) in order to have new host-vector systems certified;

IV-D-5-b-(2). Petition NIH, with notice to the IBC, for exemptions to these Guidelines (see Sections I-E-4 and I-E-5 and, for additional information on procedures, the APS); and

IV-D-5-b-(3). Petition NIH, with concurrence of the IBC, for exceptions to the prohibitions under these Guidelines (see Section I-D and, for additional information on procedures, the APS).

IV-D-5-b-(4). Petition NIH for determination of containment for

experiments requiring case-by-case review.

IV–D–5–b–(5). Petition NIH for determination of containment for experiments not covered by the Guidelines.

IV–D–5–c. *Submissions by the PI to the IBC.* The PI shall:

IV–D–5–c–(1). Make the initial determination of the required levels of physical and biological containment in accordance with the Guidelines;

IV–D–5–c–(2). Select appropriate microbiological practices and laboratory techniques to be used in the research;

IV–D–5–c–(3). Submit the initial research protocol (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

IV–D–5–c–(4). Remain in communication with the IBC throughout the conduct of the project.

IV–D–5–d. *PI Responsibilities After Approval but Prior to Initiating the Research.* The PI is responsible for:

IV–D–5–d–(1). Making available to the laboratory staff copies of the approved protocols that describe the potential biohazards and the precautions to be taken;

IV–D–5–d–(2). Instructing and training staff in the practices and techniques required to ensure safety and in the procedures for dealing with accidents; and

IV–D–5–d–(3). Informing the staff of the reasons and provisions for any precautionary medical practices advised or requested, such as vaccinations or serum collection.

IV–D–5–e. *PI Responsibilities During the Conduct of the Approved Research.* The PI is responsible for:

IV–D–5–e–(1). Supervising the safety performance of the staff to ensure that the required safey practices and techniques are employed:

IV–D–5–e–(2). Investigating and reporting in writing to ORDA, the Biological Safety Officer (where applicable), and the IBC any significant problems pertaining to the operation and implementation of containment practices and procedures;

IV–D–5–e–(3). Correcting work errors and conditions that may result in the release of recombinant DNA materials;

IV–D–5–e–(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); and

IV–D–5–e–(5). *Publications.* PIs are urged to include, in all publications reporting on recombinant DNA research, a description of the physical and biological containment procedures employed.

IV–E. *Responsibilities of NIH.*

IV–E–1. *Director.* The Director, NIH, is responsible for (i) establishing the NIH Guidelines on recombinant DNA research, (ii) overseeing their implementation, and (iii) their final interpretation.

The Director has a number of responsibilities under the Guidelines that involve the NIH Office of Recombinant DNA Activities (ORDA) and the Recombinant DNA Advisory Committee (RAC). ORDA's responsibilities under the Guidelines are administrative. Advice from the RAC is primarily scientific and technical. In certain circumstances, there is specific opportunity for public comment, with published response, before final action.

IV–E–1–a. *General Responsibilities of the Director, NIH.* The responsibilities of the Director shall include the following:

IV–E–1–a–(1). Promulgating requirements as necessary to implement the Guidelines.

IV–E–1–a–(2). Establishing and maintaining the RAC to carry out the responsibilities set forth in Section IV–E–2. The RAC's membership is specified in its charter and in Section IV–E–2;

IV–E–1–a–(3). Establishing and maintaining ORDA to carry out the responsibilities defined in Section IV–E–3; and

IV–E–1–a–(4). Maintaining the Federal Interagency Advisory Committee on Recombinant DNA Research established by the Secretary, HEW, for advice on the coordination of all Federal programs and activities relating to recombinant DNA, including activities of the RAC.

IV–E–1–b. *Specific Responsibilities of the Director, NIH.* In carrying out the responsibilities set forth in this Section, the Director shall weigh each proposed action, through appropriate analysis and consultation, to determine that it complies with the Guidelines and presents no significant risk to health or the environment.

IV–E–1–b–(1). *The Director is responsible for the following major actions* (For these, the Director must seek the advice of the RAC and provide an opportunity for public and Federal agency comment. Specifically, the agenda of the RAC meeting citing the major actions will be published in the **Federal Register** at least 30 days before the meeting, and the Director will also publish the proposed actions in the **Federal Register** for comment at least 30 days before the meeting. In addition, the Director's proposed decision, at his discretion, may be published in the **Federal Register** for 30 days for comment before final action is take. The Director's final decision, along with response to the comments, will be published in the **Federal Register** and the *Recombinant DNA Technical Bulletin.* The RAC and IBC chairpersons will be notified of this decision):

IV–E–1–b–(1)–(a). Changing containment levels for types of experiments that are specified in the Guidelines when a major action is involved;

IV–E–1–b–(1)–(b). Assigning containment levels for types of experiments that are not explicitly considered in the Guidelines when a major action is involved;

IV–E–1–b–(1)–(c). Certifying new host-vector systems, with the exception of minor modifications of already certified systems. [The standards and procedures for certification are described in Section II–D–2–a. Minor modifications constitute, for example, those of minimal or no consequence to the properties relevant to containment. See the Administrative Practices Supplement (APS) for further information];

IV–E–1–b–(1)–(d). Promulgating and amending a list of classes of recombinant DNA molecules to be exempt from these Guidelines because they consist entirely of DNA segments from species that exchange DNA by known physiological processes, or otherwise do not present a significant risk to health or the environment (see Sections I–E–4 and –5 and the APS for further information);

IV–E–1–b–(1)–(e). Permitting exceptions to the prohibited experiments in the Guidelines, in order, for example, to allow risk-assessment studies; and

IV–E–1–b–(1)–(f). Adopting other changes in the Guidelines.

IV–E–1–b–(2). *The Director is also responsible for the following lesser actions* (For these, the Director must seek the advice of the RAC. The Director's decision will be transmitted to the RAC and IBC chairpersons and published in the Recombinant DNA Technical Bulletin):

IV–E–1–b–(2)–(a). Interpreting and determining containment levels, upon request by ORDA;

IV–E–1–b–(2)–(b). Changing containment levels for experiments that are specified in the Guidelines (see Section III);

IV–E–1–b–(2)–(c). Assigning containment levels for experiments not explicitly considered in the Guidelines (see Section III);

IV–E–1–b–(2)–(d). Designating certain class 2 agents as class 1 for the purpose

of these Guidelines (see Footnote 1 and Appendix B);

IV–E–1–b–(2)–(e). Assigning containment levels for experiments with recombinant DNA from Class 3 organisms[1] and assigning containment levels for experiments which increase the host-range and virulence of plant pathogens beyond that which occurs by natural genetic exchange.

IV–E–1–b–(3). *The Director is also responsible for the following actions.* (The Director's decision will be transmitted to the RAC and IBC chairpersons and published in the *Recombinant DNA Technical Bulletin):*

IV–E–1–b–(3)–(a). Interpreting the Guidelines for experiments to which the Guidelines specifically assign containment levels;

IV–E–1–b–(3)–(b). Determining appropriate containment conditions for experiments according to case precedents developed under Section IV–E–1–b–(2)–(c).

IV–E–1–b–(3)–(c). Determining appropriate containment conditions upon case-by-case analysis of experiments explicitly considered in the Guidelines but for which no containment levels have been set (see Footnote 45 in Part V; Sections III–C–1–a through –e; and Sections III–C–2 and –3);

IV–E–1–b–(3)–(d). Authorizing, under procedures specified by the RAC, large-scale experiments (i.e., involving more than 10 liters of culture) for recombinant DNAs that are rigorously characterized and free of harmful sequences (see Footnote 3 and Section I–D–6);

IV–E–1–b–(3)–(e). Lowering containment levels for characterized clones or purified DNA (see Sections III–A–3–a and –b, Footnotes 3 and 41);

IV–E–1–b–(3)–(f). Approving minor modifications of already certified host-vector systems. (The standards and procedures for such modifications are described in Section II–D–2); and

IV–E–1–b–(3)–(g). Decertifying already certified host-vector systems.

IV–E–1–b–(3)–(h). Assigning containment levels for experiments in which both donor and recipient are nonpathogenic prokaryotes and/or nonpathogenic lower eukaryotes (see Section III–0–2).

IV–E–1–b–(3)–(i). Adding new entries to the list of toxins for vertebrates (see Appendix G).

IV–E–1–b–(3)–(j). Approving the cloning of toxin genes in host-vector systems other than *E. coli* K–12 (see Appendix G).

IV–E–1–b–(4). The Director shall conduct, support, and assist training programs in laboratory safety for Institutional Biosafety Committtee members, Biological Safety Officers, Principal Investigators, and laboratory staff.

IV–E–1–b–(5). The Director, at the end of 36 months from the time these Guidelines are promulgated, will report on the Guidelines, their administration, and the potential risks and benefits of this research. In doing so, the Director will consult with the RAC and the Federal Interagency Committee. Public comment will be solicited on the draft report and taken into account in transmitting the final report to the Assistant Secretary for Health and the Secretary, HHS.

IV–E–2. *Recombinant Advisory Committee.* The NIH Recombinant DNA Advisory Committee (RAC) is responsible for carrying out specified functions cited below as well as others assigned under its charter or by the Secretary, HHS, the Assistant Secretary for Health, and the Director, NIH.

The members of the committee shall be chosen to provide, collectively expertise in scientific fields relevant to recombinant DNA technology and biological safety—e.g., microbiology, molecular biology, virology, genetics, epidemiology, infectious diseases, the biology of enteric organisms, botany, plant pathology, ecology, and tissue culture. At least 20 percent of the members shall be persons knowledgeable in applicable law, standards of professional conduct and practice, public attitudes, the environment, public health, occupational health, or related fields. Representatives from Federal agencies shall serve as nonvoting members. Nominations for the RAC may be submitted to the NIH Office of Recombinant DNA Activities, Bethesda, Md., 20205.

All meetings of the RAC will be announced in the **Federal Register**, including tentative agenda item, 30 days in advance of the meeting, with final agendas (if modified) available at least 72 hours before the meeting. No item defined as a major action under Section IV–E–1–b–(1) may be added to an agenda after it appears in the **Federal Register.**

IV–E–2–a. *The RAC shall be responsible for advising the Director, NIH, on the actions listed in Section IV–E–1–b–(1) and (2).*

IV–E–3. *The Office of Recombinant DNA Activities.* ORDA shall serve as a focal point for information on recombinant DNA activities and provide advice to all within and outside NIH, including Institutions, Biological Safety Committees, Principal Investigators, Federal agencies, State and local governments, and institutions in the private sector. ORDA shall carry out such other functions as may be delegated to it by the Director, NIH, including those authorities described in Section IV–E–1–b–(3). In addition, ORDA shall be responsible for the following:

IV–E–3–a. Review and approval of Institutional Biosafety Committee (IBC) membership;

IV–E–3–b through IV–E–3–c–(3). [Deleted]

IV–E–c–(4). Publish in the **Federal Register:**

IV–E–c–(4)–(a). Announcements of Recombinant DNA Advisory Committee (RAC) meetings and agendas 30 days in advance, with publication of the Director's proposed decision for 30 days of public and Federal agency comment followed by a published response, on any action listed in Section IV–E–1–(b)–(1); and

IV–E–3–c–(4)–(b). Announcements of RAC meetings and agendas 30 days in advance of any action listed in Section IV–E–1–b–(2).

Note.—If the agenda for an RAC meeting is modified, ORDA shall make the revised agenda available to anyone, upon request, at least 72 hours in advance of the meeting.

IV–E–3–c–(5). Publish the *Recombinant DNA Technical Bulletin;* and

IV–E–3–c–(6). Serve as executive secretary to the RAC.

IV–E–4. *Other NIH Components.* Other NIH components shall be responsible for:

IV–E–4–a. [Deleted]

IV–E–4–b. Certifying P4 facilities, inspecting them periodically, and inspecting other recombinant DNA facilities as deemed necessary; and

IV–E–4–c. Announcing and distributing certified HV2 and HV3 host-vector systems (see Section II–E–3).

(See Administrative Practices Supplement for additional information on the administrative procedures of ORDA and other NIH components.)

IV–F. [Deleted]

IV–G. *Compliance.* As a condition for NIH funding or recombinant DNA research, Institutions must ensure that such research conducted at or sponsored by the Institution, irrespective of the source of funding, shall comply with these Guidelines. The policies on noncompliance are as follows:

IV–G–1. All NIH-funded projects involving recombinant DNA techniques must comply with the NIH Guidelines. Noncompliance may result in (i) suspension, limitation, or termination of financial assistance for such projects and of NIH funds for other recombinant DNA research at the Institution, or (ii) a

requirement for prior NIH approval of any or all recombinant DNA projects at the Institution.

IV–G–2. All non-NIH funded projects involving recombinant DNA techniques conducted at or sponsored by an Institution that receives NIH funds for projects involving such techniques must comply with the NIH Guidelines. Noncompliance may result in (i) suspension, limitation, or termination of NIH funds for recombinant DNA research at the Institution, or (ii) a requirement for prior NIH approval of any or all recombinant DNA projects at the Institution.

IV–G–3. Information concerning noncompliance with the Guidelines may be brought forward by any person. It should be delivered to both NIH (ORDA) and the relevant Institution. The Institution, generally through the IBC, shall take appropriate action. The Institution shall forward a complete report of the incident to ORDA, recommending any further action indicated.

IV–G–4. In cases where NIH proposes to suspend, limit, or terminate financial assistance because of noncompliance with the Guidelines, applicable DHEW and Public Health Service procedures shall govern.

IV–G–5. *Voluntary Compliance.* Any individual, corporation, or institution that is not otherwise covered by the Guidelines is encouraged to conduct recombinant DNA research activities in accordance with the Guidelines, through the procedures set forth in Part VI.

V. Footnotes and References

(*1*) The reference to organisms as Class 1, 2, 3, 4, or 5 refers to the classification in the publication *Classification of Etiologic Agents on the Basis of Hazard,* 4th Edition, July 1974; U.S. Department of Health, Education, and Welfare, Public Health Service, Centers for Disease Control, Office of Biosafety, Atlanta, Georgia 30333. The list of organisms in each class, as given in this publication, is reprinted in Appendix B to these Guidelines.

The Director, NIH, with advice of the Recombinant DNA Advisory Committee, may designate certain of the agents which are listed as Class 2 in the *Classification of Etiologic Agents on the Basis of Hazard,* 4th Edition, July 1974, as Class 1 agents for the Purposes of these Guidelines (see Section IV–E–1–b–(2)–(d)). An updated list of such agents may be obtained from the Office of Recombinant DNA Activities (ORDA), National Institutes of Health, Bethesda, Maryland 20205.

The entire *Classification of Etiologic Agents on the Basis of Hazard* is in the process of revision.

(*2*) For experiments using Vesicular Stomatitis virus (VSV), contact the NIH Office of Recombinant DNA Activities.

(*2A*) In Parts I and III of the Guidelines, there are a number of places where judgments are to be made. These include: "cells known to be infected with such agents" (Section I–D–1); "known to acquire it naturally" (Section I–D–5); "known to produce a potent polypeptide toxin * * * or known to carry such pathogens * * * not likely to be a product of closely linked eukaryote genes * * * shown not to contain such agents" (Section III–A–1–a–(5)–(a)); "shown to be free of disease causing microorganisms" (Section III–A–1–a(5)–(b)); "close relatives" (Section III–C–3); and "produce a potent polypeptide toxin" (Footnote 34).

In all these cases the principal investigator is to make the initial judgment on these matters as part of his responsibility to "make the initial determination of the required levels of physical and biological containment in accordance with the Guidelines" (Section IV–D–7–a). In all these cases, this judgment is to be reviewed and approved by the Institutional Biosafety Committee as part of its responsibility to make "an independent assessment of the containment levels required by these Guidelines for the proposed research" (Section IV–D–3–a–(1)). If the IBC wishes, any specific cases may be referred to the NIH Office of Recombinant DNA Activities as part of ORDA's functions to "provide advice to all within and outside NIH" (Section IV–E–3), and ORDA may request advice from the Recombinant DNA Advisory Committee as part of the RAC's responsibility for "interpreting and determining containment levels upon request by ORDA" (Section IV–E–1–b–(2)–(a)).

(*3*) The following types of data should be considered in determining whether DNA recombinants are "characterized" and the absence of harmful sequences has been established: (a) The absence of potentially harmful genes (e.g., sequences contained in indigenous tumor viruses or sequences that code for toxins, invasins, virulence factors, etc., that might potentiate the pathogenicity or communicability of the vector and/or the host or be detrimental to humans, animals, or plants); (b) the type(s) of genetic information on the cloned segment and the nature of transcriptional and translation gene products specified; (c) the relationship between the recovered and desired segment (e.g., hybridization and restriction endonuclease fragmentation analysis where applicable); (d) the genetic stability of the cloned fragment; and (e) any alterations in the biological properties of the vector and host.

(*4*) In Section I–E, "exemptions" from the Guidelines are discussed. Such experiments are not covered by the Guidelines and need not be registered with NIH. In Section I–D on "prohibitions," the possibility of "exceptions" is discussed. An "exception" means that an experiment may be expressly released from a prohibition. At that time it will be assigned an appropriate level of physical and biological containment.

(*5*) Care should be taken to inactivate recombinant DNA before disposal. Procedures for inactivating DNA can be found in the "Laboratory Safety Monograph: A Supplement to the NIH Guidelines for Recombinant DNA Research."

(*6*) *Laboratory Safety at the Center for Disease Control* (Sept. 1974). U.S. Department of Health Education and Welfare Publication No. CDC 75–8118.

(*7*) *Classification of Etiologic Agents on the Basis of Hazard.* (4th Edition, July 1974). U.S. Department of Health, Education and Welfare. Public Health Service. Centers for Disease Control, Office of Biosafety, Atlanta, Georgia 30333.

(*8*) *National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses* (Oct. 1974). U.S. Department of Health, Education and Welfare Publication No. (NIH) 75–790.

(*9*) *National Institutes of Health Biohazards Safety Guide* (1974). U.S. Department of Health, Education, and Welfare, Public Health.

(*10*) *Biohazards in Biological Research* (1973). A. Hellman, M. N. Oxman, and R. Pollack (ed.) Cold Spring Harbor Laboratory.

(*11*) *Handbook of Laboratory Safety* (1971). Second Edition. N. V. Steere (ed.). The Chemical Rubber Co., Cleveland.

(*12*) Bodily, J. L. (1970). *General Administration of the Laboratory,* H. L. Bodily, E. L. Updyke, and J. O. Mason (eds.), Diagnostic Procedures for Bacterial Mycotic and Parasitic Infections. American Public Health Association, New York, pp. 11–28.

(*13*) Darlow, H. M. (1969). *Safety in the Microbiological Laboratory.* In J. R. Norris and D. W. Robbins (ed.), Methods in Microbiology, Academic Press, Inc., New York. pp. 169–204.

(*14*) *The Prevention of Laboratory Acquired Infection* (1974). C. H. Collins, E. G. Hartley, and R. Pilsworth. Public Health Laboratory Service, Monograph Series No. 6.

(*15*) Chatigny, M. A. (1961). *Protection Against Infection in the Microbiological Laboratory: Devices and Procedures.* In W. W. Umbreit (ed.). Advances in Applied Microbiology. Academic Press, New York, N.Y. 3:131–192.

(*16*) *Design Criteria for Viral Oncology Research Facilities* (1975). U.S. Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, DHEW Publication No. (NIH) 75–891.

(*17*) Kuehne, R. W. (1973). *Biological Containment Facility for Studing Infectious Disease.* Appl. Microbiol. 26–239–243.

(*18*) Runkle, R. S., and G. B. Phillips (1969). *Microbial Containment Control Facilities.* Van Nostrand Reinhold, New York.

(*19*) Chatigny, M. A., and D. I. Clinger (1969). *Contamination Control in Aerobiology.* In R. L. Dimmick and A. B. Akers (eds.). An Introduction to Experimental Aerobiology. John Wiley & Sons, New York, pp. 194–263.

(*19A*) Horsfall, F. L., Jr., and J. H. Baner (1940). *Individual Isolation of Infected Animals in a Single Room.* J. Bact. *40,* 569–580.

(*20*) Biological safety cabinets referred to in this section are classified as *Class I, Class II,* or *Class III* cabinets. A *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. A *Class II* cabinet 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. Design and performance specifications for *Class II* cabinets have been adopted by the National Sanitation Foundation, Ann Arbor, Michigan. A *Class III* cabinet 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 contaiminants 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 discharged to the outside environment.

(*21*) Hershfield, V., H. W. Boyer, C. Yanofsky, M. A. Lovett, and D. R. Helinski (1974). *Plasmid ColEl as a Molecular Vehicle for Cloning and Amplification of DNA.* Proc. Nat. Acad. Sci. USA *71,* 3455–3459.

(*22*) Wensink, P. C., D. J. Finnegan, J. E. Donelson, and D. S. Hogness (1974). *A System for Mapping DNA Sequences in the Chromosomes of Drosophila Melanogaster.* Cell *3,* 315–335.

(*23*) Tanaka, T., and B. Weisblum (1975). *Construction of a Colicin El–R Factor Composite Plasmid In Vitro: Means for Amplification of Deoxyribonucleic Acid.* J. Bacteriol. *121,* 354–362.

(*24*) Armstrong, K. A., V. Hershfield, and D. R. Helinski (1977). *Gene Cloning and Containment Properties of Plasmid Col El and Its Derivatives,* Science *196,* 172–174.

(*25*) Bolivar, F., R. L. Rodriguez, M. C. Betlach, and H. W. Boyer (1977). *Construction and Characterization of New Cloning Vehicles: I. Ampicillin-Resistant Derivative of pMB9.* Gene *2,* 75–93.

(*26*) Cohen, S. N., A. C. W. Chang, H. Boyer, and R. Helling (1973). *Construction of Biologically Functional Bacterial Plasmids in Vitro.* Proc. Natl. Acad. Sci. USA 70, 3240–3244.

(*27*) Bolivar, F., R. L. Rodriquez, R. J. Greene, M. C. Batlach, H. L. Reyneker, H. W. Boyer, J. H. Crosa, and S. Falkow (1977). *Construction and Characterization of New Cloning Vehicles: II. A Multi-Purpose Cloning System.* Gene *2,* 95–113.

(*28*) Thomas, M., J. R. Cameron, and R. W. Davis (1974). *Viable Molecular Hybrids of Bacteriophage Lambda and Eukaryotic DNA.* Proc. Nat. Acad. Sci. USA *71,* 4579–4583.

(*29*) Murray, N.E., and K. Murray (1974). *Manipulation of Restriction Targets in Phage Lambda to Form Receptor Chramosomes for DNA Fragments.* Nature *251,* 476–481.

(*30*) Rambach, A., and P. Tiollais (1974). *Bacteriophage Having EcoRI Endonuclease Sites Only in the Non-Essential Region of the Genome.* Proc. Nat. Acad. Sci., USA *71,* 3927–3930.

(*31*) Blattner, F. R., B. G. Williams, A. E. Bleche, K. Denniston-Thompson, H. E. Faber, L. A. Furlong, D. J. Gunwald, D. O. Kiefer, D. D. Moore, J. W. Shumm, E. L. Sheldon, and O. Smithies (1977). *Charon Phages: Safer Derivatives of Bacteriophage Lambda for DNA Cloning.* Science *196,* 163–169.

(*32*) Donoghue, D. J., and P. A. Sharp (1977). *An Improved Lambda Vector: Construction of Model Recombinants Coding for Kanamycin Resistance,* Gene *1,* 209–227.

(*33*) Leder, P., D. Tiemeier and L. Enquist (1977). *EK2 Derivatives of Bacteriophage Lambda Useful in the Cloning of DNA from Higher Organisms: The gt WES System.* Science *196.* 175–177.

(*33A*) Skalka, A. (1978). *Current Status of Coliphage EK2 Vectors.* Geen *3,* 29–35.

(*33B*) Szybalski, W., A. Skalka, S. Gottesman, A. Campbell, and D. Botstein (1978). *Standardized Laboratory Tests for EK2 Certification.* Gene *3,* 36–38.

(*34*) We are specifically concerned with the remote possibility that potent toxins could be produced by acquiring a single gene or cluster of genes. See also footnote 2A.

(*35*) Defined as observable under optimal laboratory conditions by transformation, transduction, phage infection, and/or conjugation with transfer of phage, plasmid, and/or chromosomal genetic information. Note that this definition of exchange may be less stringent than that applied to exempt organisms under Section I–E–4.

(*36*) As classified in the Third Report of the International Committee on Taxonomy of Viruses: Classification and Nomenclature of Viruses, R. E. F. Matthews, Ed. Intervirology 12 (129–296) 1979. (As noted in the Prohibition Section, the use of viruses classified[1] as Class 4 or 5 is prohibited.)

(*37*) The cDNA copy of the viral mRNA must be ⩾99% pure; otherwise as for shotgun experiments with eukaryotic cellular DNA.

(*37A*) For the purpose of these Guidelines, viruses of the families *Papovaviridae*, *Adenoviridae*, and *Herpetoviridae* (36) should be considered as "transforming" viruses. While only certain of these viruses have been associated with cell transformation *in vivo* or *in vitro,* it seems prudent to consider all members to be potentially capable of transformation. In addition, those viruses of the family *Poxviridae* that produce proliferative responses—i.e., myxoma, rabbit and squirrel fibroma, and Yaba viruses—should be considered as "transforming."

(*38*) ⩾99% pure (i.e., less than 1% of the DNA consists of intact viral genomes); otherwise as for whole genomes.

(*39*) The viruses have been classified by NCI as "moderate-risk oncogenic viruses." See "Laboratory Safety Monograph—A Supplement to the NIH Guidelines for Recombinant DNA Research" for recommendations on handling the viruses themselves.

(*40*) [Deleted]

(*41*) The DNA preparation is defined as "purified" if the desired DNA represents at least 99% (w/w) of the total DNA in the preparation, provided that it was verified by more than one procedure.

(*42*) The lowering of the containment level when this degree of purification has been obtained is based on the fact that the total number of clones that must be examined to obtain the desired clone is markedly reduced. Thus, the probability of cloning a harmful gene could, for example, be reduced by more than 10^5-fold when a nonrepetitive gene from mammals was being sought. Furthermore, the level of purity specified here makes it easier to establish that the desired DNA does not contain harmful genes.

(*43*) This is not permitted, of course, if it falls under any of the Prohibitions of Section I–D. Of particular concern here is prohibition I–D–5, i.e., "Deliberate transfer of a drug resistance trait to micro-organisms that are not known to acquire it naturally if such acquisition could compromise the use of a drug to control disease agents in human or veterinary medicine or agriculture."

(*44*) Because this work will be done almost exclusively in tissue culture cells, which have no capacity for propagation outside the laboratory, the primary focus for containment is the vector. It should be pointed out that risk of laboratory-acquired infection as a consequence of tissue culture manipulation is very low. Given good microbiological practices, the most likely mode of escape of recombinant DNAs from a physically contained laboratory is carriage by an infected human. Thus the vector with an inserted DNA segment should have little or no ability to replicate or spread in humans.

For use as a vector in a vertebrate host cell system, an animal viral DNA molecule should display the following properties:

(i) It should not consist of the whole genome of any agent that is infectious for humans or that replicates to a significant extent in human cells in tissue culture. If the recombinant molecule is used to transform nonpermissive cells (i.e., cells which do not produce infectious virus particles), this is not a requirement.

(ii) It should be derived from a virus whose epidemiological behavior and host range are well understood.

(iii) In permissive cells, it should be defective when carrying an inserted DNA segment (i.e., propagation of the recombinant DNA as a virus must be dependent upon the presence of a complementing helper genome). In almost all cases this condition would be achieved automatically by the manipulations used to construct and propagate the recombinants. In addition, the amount of DNA encapsidated in the particles of most animal viruses is defined within fairly close limits. The insertion of sizable foreign DNA sequences, therefore, generally demands a compensatory deletion of viral sequences. It may be possible to introduce very short insertions (50–100 base pairs) without rendering the viral vector defective. In such a situation, the requirement that the viral vector be defective is not necessary, except in those cases in which the inserted DNA encodes a biologically active polypeptide.

It is desired but not required that the functional anatomy of the vector be known—that is, fL-re sLogld be a clear idea of the location within the molecule of:

(i) The sites at which DNA synthesis originates and terminates,

(ii) The sites that are cleaved by restriction endonucleases and

(iii) The template regions for the major gene product.

If possible the helper virus genome should:

(i) Be integrated into the genome of a stable line of host cells (a situation that would effectively limit the growth of the vector recombinant to such cell lines) or

(ii) Consist of a defective genome, or an appropriate conditional lethal mutant virus, making vector and helper dependent upon each other for propagation.

However, neither of these stipulations is a requirement.

(*45*) Review by NIH on a case-by-case basis means that NIH must review and set appropriate containment conditions before the work may be undertaken. NIH actions in such case-by-case reviews will be published in the *Recombinant DNA Technical Bulletin.*

(*46*) Provided the inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated on a case-by-case basis.

(*47*) ≥99% pure; otherwise as for shotgun experiments.

(*48*) A USDA permit, required for import and interstate transport of pathogens, may be obtained from the Animal and Plant Health Inspection Service, USDA, Federal Building, Hyattsville, MD 20782.

(*49*) A subset of non-conjugative plasmid vectors are also poorly mobilizable (e.g., pBR322, pBR313). Where practical, these vectors should be employed.

(*50*) i.e., the total of all genomes within a Family shall not exceed two-thirds of the genome.

VI. Voluntary Compliance

VI–A. *Basic Policy.* Individuals, corporations, and institutions not otherwise covered by the Guidelines are encouraged to do so by following the standards and procedures set forth in Parts I–IV or the Guidelines. In order to simplify discussion, references hereafter to "institutions" are intended to encompass corporations, and individuals who have no organizational affiliation. For purposes of complying with the Guidelines, an individual intending to carry out research involving recombinant DNA is encouraged to affiliate with an institution that has an Institutional Biosafety Committee approved under the Guidelines.

Since commercial organizations have special concerns, such as protection of proprietary data, some modifications and explanations of the procedures in Parts I–IV are provided below, in order to address these concerns.

VI–B. *IBC Approval.* The NIH Office of Recombinant DNA Activities (ORDA) will review the membership of an institution's Institutional Biosafety Committee (IBC) and, where it finds the IBC meets the requirements set forth in Section IV–D–2, will give its approval to the IBC membership.

It should be emphasized that employment of an IBC member solely for purposes of membership on the IBC does not itself make the member an institutionally affiliated member for purposes of Section IV–D–2–a.

Except for the unaffiliated members, a member of an IBC for an institution not otherwise covered by the Guidelines may participate in the review and approval of a project in which the member has a direct financial interest, so long as the member has not been and does not expect to be engaged in the project. Section IV–D–2–d is modified to that extent for purposes of these institutions.

VI–C. [Deleted]

VI–D. *Certification of Host-Vector Systems.* A host-vector system may be proposed for certification by the Director, NIH, in accordance with the procedures set forth in Section II–D–2–a.

Institutions not otherwise covered by the Guidelines will not be subject to Section II–D–3 by complying with these procedures.

In order to ensure protection for proprietary data, any public notice regarding a host-vector system which is designated by the institution as proprietary under Section VI–F–1 will be issued only after consultation with the institution as to the content of the notice.

VI–E. *Requests for Exceptions, Exemptions, Approvals.* Requests for exceptions from prohibitions, exemptions, or other approvals required by the Guidelines should be requested by following the procedures set forth in the appropriate sections in Parts I–IV of the Guidelines.

In order to ensure protection for proprietary data, any public notice regarding a request for an exception, exemption, or other approval which is designated by the institution as proprietary under Section VI–F–1 will be issued only after consultation with the institution as to the content of the notice.

VI–F. *Protection of Proprietary Data.* In general, the Freedom of Information Act requires Federal agencies to make their records available to the public upon request. However, this requirement does not apply to, among other things, "trade secrets and commercial and financial information obtained from a person and privileged or confidential." 18 U.S.C. 1905, in turn makes it a crime for an officer or employee of the United States or any Federal department or agency to publish, divulge, disclose, or make known "in any manner or to any extent not authorized by law any information coming to him in the course of his employment or official duties or by reason of any examination or investigation made by or return, report or record made to or filed with, such department or agency or officer of employee thereof, which information concerns or relates to the trade secrets, (or processes * * * of any person, firm, partnership, corporation, or association," This provision applies to all employees of the Federal Government, including special Government employees. Members of the Recombinant DNA Advisory Committee are "special Government employees."

VI–F–1. In submitting information to NIH for purposes of complying voluntarily with the Guidelines, an institution may designate those items of information which the institution believes constitute trade secrets or privileged or confidential commercial or financial information.

VI–F–2. If NIH receives a request under the Freedom of Information Act for information so designated, NIH will promptly contact the institution to secure its views as to whether the information (or some portion) should be released.

V–F–3. If the NIH decides to release this information (or some portion) in response to a Freedom of Information request or otherwise, the institution will be advised; and the actual release will not be made until the expiration of 15 days after the institution is so advised, except to the extent that earlier release, in the judgement of the Director, NIH, is necessary to protect against an imminent hazard to the public or the environment.

VI–F–4. Projects should be registered in accordance with procedures specified in the *Administrative Practices Supplement.* The following information will usually be considered publicly available information, consistent with the need to protect proprietary data:

a. The names of the institution and principal investigator.

b. The location where the experiments will be performed.

c. The host-vector system.

d. the source of the DNA.

e. The level of physical containment.

VI–F–5–a. Any institution not otherwise covered by the Guidelines, which is considering submission of data or information voluntarily to NIH, may request presubmission review of the records involved to determine whether, if the records are submitted, NIH will or will not make part or all of the records available upon request under the Freedom of Information Act.

VI–F–5–b. A request for presubmission review should be submitted to ORDA, along with the records involved. These records must be clearly marked as being the property of the institution, on loan to NIH solely for the purpose of making a determination under the Freedom of Information Act. ORDA will then seek a determination from the HEW Freedom of Information Officer, the responsible official under HEW regulations (45 CFR Part 5), as to whether the records involved (or some portion) are or are not available to members of the public under the Freedom of Information Act. Pending such a determination, the records will be kept separate from ORDA files, will be considered records of the institution and not ORDA, and will not be received as part of ORDA files. No copies will be made of the records.

VI–F–5–c. ORDA will inform the institution of the HEW Freedom of Information Officer's determination and follow the institution's instructions as to where some or all of the records involved are to be returned to the institution or to become a part of ORDA files. If the institution instructs ORDA to return the records, no copies or summaries of the records will be made or retained by HEW, NIH, or ORDA.

VI–F–5–d. The HEW Freedom of Information Officer's determination will represent the official's judgment, as of the time of the determination, as to whether the records involved (or some portion) would be exempt from disclosure under the Freedom of Information Act, if at the time of the determination the records were in ORDA files and a request were received from them under the Act.

Appendix A of Annex E.—Exemptions Under I–E–4

Section I–E–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. A list of such exchangers will be prepared and periodically revised by the Director, NIH, with advice of the Recombinant DNA Advisory Committee, after appropriate notice and opportunity for public comment (see Section IV–E–1–b–(1)–(d).) Certain classes are exempt as of publication of these Revised Guidelines. The list is in Appendix A."

Under exemption I–E–4 of these revised 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*, eighth edition. R.E. Buchanan and N.E. Gibbons, editors. Williams and Wilkins Company: Baltimore, 1974.)

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 *seudomonas fluorescens*
9. *Serratia marcescens*

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

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*

Appendix B of Annex E.—Classification of Microorganisms on the Basis of Hazard

I. *Classification of Etiologic Agents on the Basis of Hazard (1)*

A. Class 1 Agents

All bacterial, parasitic, fungal, viral, rickettsial, and chlamydial agents not included in higher classes.

B. Class 2 Agents

1. Bacterial Agents

Actinobacillus—all species except *A. mallei*, which is in Class 3
Arizona hinshawii—all serotypes
Bacillus anthracis
Bordetella—all species
Borrelia recurrentis, B. vincenti
Clostridium botulinum, Cl. chauvoei, Cl. haemolyticum, Cl. histolyticum, Cl. novyi, Cl. septicum, Cl. tetani
Corynebacterium diptheriae, C. equi, C. haemolyticum, C. pseudotuberculosis, C. pyogenes, C. renale
Diplococcus (Streptococcus) pneumoniae
Erysipelothrix insidiosa
Escherichia coli—all enteropathogenic serotypes
Haemophilus ducreyi, H. influenzae
Herellae vaginicola
Klebsiella—all species and all serotypes
Leptospira interrogans—all serotypes
Listeria—all species
Mima polymorpha
Moraxella—all species
Mycobacteria—all species except those listed in Class 3
Mycoplasma—all species except *Mycoplasma mycoides* and *Mycoplasma agalactiae*, which are in Class 5
Neisseria gonorrhoeae, N. meningitidis
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 pyogenes
Treponema carateum, T. pallidum, and T. pertenue
Vibrio fetus, V. comma, including biotype El Tor, and *V. parahemolyticus*

2. Fungal Agents

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

3. Parasitic Agents

Endamoeba histolytica
Leishmania sp.
Naegleria gruberi
Toxoplasma gondii
Toxocara canis
Trichinella spiralis
Trypanosoma cruzi

4. Viral, Rickettsial, and Chlamydial Agents

Adenoviruses—human—all types
Cache Valley virus
Coxsackie A and B viruses
Cytomegaloviruses
Echoviruses—all types.
Encephalomyocarditis virus (EMC)
Flanders virus
Hart Park virus
Hepatitis-associated antigen material
Herpes viruses—except *Herpesvirus simiae* (Monkey B virus) which is in Class 4
Coroma 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, Monkey pox, and Whitepox,* which depending on experiments, are in Class 3 or Class 4
Rabies virus—all strains except *Rabies street virus,* which should be classified in Class 3 when inoculated into carnivores
Reoviruses—all types
Respiratory syncytial virus
Rhinoviruses—all types
Rubella virus
Simian viruses—all types except *Herpesvirus simiae (Monkey B virus) and Marburg virus,* which are in Class 4
Sindbis virus
Tensaw virus
Turlock virus
Vaccinia virus
Varicella virus
Volé rickettsia
Yellow fever virus, 17D vaccine strain

C. Class 3 Agents

1. Bacterial Agents

*Actinobacillus mallei**
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 pseudomallei**
Yersenia pestis

2. Fungal Agents

Coccidioides immitis
Histoplasma capsulatum
Histoplasma capsulatum var. *duboisii*

3. Parasitic Agents

Schistosoma mansoni

4. Viral, Rickettsial, and Chlamydial Agents

****Alastrim, Smallpox, Monkey pox, and Whitepox,* when used *in vitro*
Arboviruses—all strains except those in Classes 2 and 4 (Arboviruses indigenous to the United States are in Class 3, except those listed in Class 2. *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)
Psittacosis-Ornithosis-Trachoma group of agents
Rabies street virus, when used in inoculations of carnivores (See Class 2)
Rickettsia—all species except *Vole rickettsia* when used for transmission or animal inoculation experiments
*Vesicular stomatitis virus**
Yellow fever virus—wild, when used *in vitro*

D. Class 4 Agents

1. Bacterial Agents

None

3. Fungal Agents

None

3. Parasitic Agents

None

Viral, Rickettsial, and Chlamydial Agents

****Alastrim, Smallpox, Monkey pox, and Whitepox,* 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

II. Classification of Oncogenic Viruses on the Basis of Potential Hazard (2)

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

B. Moderate-Risk Oncogenic Viruses

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

III. Animal Pathogens (3)

A. Animal disease organisms which are forbidden entry into the United States by Law (CDC Class 5 agents)

1. Foot and mouth disease virus

B. Animal disease organisms and vectors which are forbidfden entry into the United States by USDA Policy (CDC Class 5 Agents)

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 evansi
Theileria parva (East Coast fever)
Theileria annulata
Theileria lawrencei
Theileria bovis
Theileria hirci
Vesicular exanthema virus
Wesselsbron disease virus
Zyonema

Footnotes and References of Appendix B

*A USDA permit, required for import and interstate commerce of pathogens, may be obtained from the Animal and Plant Health Inspection Service, USDA, Federal Building, Hyattsville, MD. 20782.

**Since the publication of the classification in 1974 [1], the *Actinomycetes* have been reclassified as bacterial rather than fungal agents.

***All activities, including storage of variola and whitepox are restricted to the single national facility (World Health Organization (WHO) Collaborating Center for Smallpox Research, Center for Disease Control, in Atlanta).

(*1*) *Classification of Etiologic 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.

(*2*) *National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses* (October 1974). U.S. Department of Health, Education, and Welfare Publication No. (NIH) 75–790

(*3*) U.S. Department of Agriculture, Animal and Plant Health Inspection Service.

Appendix C of Annex E.—Exemptions under I–E–5

Section I–E–5 states that exempt from these Guidelines are "Other classes of recombinant DNA molecules, if the Director, NIH, with advice of the Recombinant DNA Advisory Committee, after appropriate notice and opportunity for public comment, finds that they do not present a significant risk to health or the environment. (See Section IV–E–1–b–(1)–(d).) Certain classes are exempt as of publication of these Revised Guidelines."

The following classes of experiments are exempt under Section I–E–5 of the Guidelines:

1. *Recombinant DNAs in Tissue Culture.*

Recombinant DNA molecules derived entirely from non-viral components (that is, no component is derived from a eukaryotic virus), that are propagated and maintained in cells in tissue culture are exempt from these Guidelines with the exceptions listed below.

Exceptions.

Experiments described in Sections I–D–1 to I–D–5 as being prohibited.

Experiments involving DNA from Class 3 organisms [1] or cells known to be infected with these agents, or any recombinant DNA molecules which increase the virulence and host-range of a plant pathogen beyond that which occurs by natural genetic exchange. (See Section III–O–1.)

Experiments involving the deliberate introduction of genes coding for the biosynthesis of toxins potent for vertebrates. (See Appendix G.)

2. *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 (a) the *E. coli* host shall not contain conjugation proficient plasmids or generalized transducing phages, and (b) lambda or lambdoid or Ff bacteriophages or nonconjugative plasmids [49] shall be used as vectors. However, experiments involving the insertion into *E. coli* K–12 of DNA from prokaryotes that exchange genetic information [35] with *E. coli* may be performed with any *E. coli* K–12 vector (e.g., conjugative 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 experiments, P1 physical containment conditions are recommended.

Exceptions.

Experiments described in Sections I–D–1 to I–D–5 as being prohibited.

Experiments involving DNA from Class 3 organisms [1] or from cells known to be infected with these agents may be conducted at P3 containment. Lower containment levels may be specified by NIH. (See Section IV–E–1–b–(2)–(e).) Experiments in this category require prior IBC review and approval.

Experiments which increase the virulence and host range of a plant pathogen beyond that which occurs by natural genetic exchange. (See Section III–O–1.)

Large-scale experiments (e.g., more than 10 liters of culture) require prior IBC review and approval.

Experiments involving the deliberate cloning of genes coding for the biosynthesis of toxins potent for vertebrates. (See Appendix G.)

3. *Experiments Involving Saccharomyces cerevisiae host-vector systems.*

Experiments which use *Saccharomyces cerevisiae* host-vector systems, with the exception of experiments listed below, are exempt from these Guidelines provided that laboratory strains are used.

For these exempt experiments, P1 physical containment conditions are recommended.

Exceptions.

Experiments described in Sections I–D–1 to I–D–5 as being prohibited.

Experiments involving CDC Class 3 organisms [1] or cells known to be infected with these agents, or any recombinant DNA molecules which increase the virulence and host-range of a plant pathogen beyond that which occurs by natural genetic exchange. (See Section III–O–1.)

Large-scale experiments (e.g., more than 10 liters of culture) require prior IBC review and approval.

Experiments involving the deliberate cloning of genes coding for the biosynthesis of toxins potent for vertebrates. (See Appendix G.)

4. *Experiments Involving Bacillus subtilis host-vector systems.*

Any asporogenic *Bacillus subtilis* strain which does not revert to a sporeformer with a frequency greater than 10^{-7} can be used for cloning DNA from any nonprohibited source, with the exception of those experiments listed below. Indigenous *Bacillus* plasmids and phages, whose host-range does not include *Bacillus cereus* or *Bacillus anthracis*, may be used as vectors.

For these exempt experiments P1 physical containment conditions are recommended.

Exceptions.

Experiments described in Section I–D–1 to I–D–5 as being prohibited.

Experiments involving CDC Class 3 organisms [1] or cells known to be infected with these agents, or any recombinant DNA molecules which increase the virulence and host-range of a plant pathogen beyond that which occurs by natural genetic exchange. (See Section III–O–1.)

Large-scale experiments (e.g., more than 10 liters of culture) require prior IBC review and approval.

Experiments involving the deliberate cloning of genes coding for the biosynthesis of toxins potent for vertebrates. (See Appendix G.)

Appendix D of Annex E.—HV1 and HV2 Host-Vector Systems Assigned Containment Levels as Specified in the Subsections of Section III–A

As noted above at the beginning of Section III–A, certain HV1 and HV2 host-vector systems are assigned containment levels as specified in the subsections of Section III–A. Those so classified as of publication of these Revised Guidelines are listed below.

*HV1—The following specified strains of *Neurospora crassa* which have been modified to prevent aerial dispersion:

(1) inl (inositolless) strains 37102, 37401, 46316, 64001 and 89601.

(2) csp–1 strain UCLA37 and csp–2 strains FS 590, UCLA101 (these are conidial separation mutants).

(3) eas strain UCLA191 (an "easily wettable" mutant).

HV1—The following *Streptomyces* species: *Streptomyces coelicolor, S. lividans, S. parvulus,* and *S. griseus.* The following are accepted as vector components of certified *Streptomyces* HV1 systems: *Streptomyces* plasmids SCP2, SLP1.2, pIJ101, actinophage phi C31, and their derivatives.

Appendix E of Annex E.—Actions Taken Under the Guidelines

As noted in the subsections of Sections IV–E–1–b–(1) and IV–E–1–b–(2), the Director, NIH, may take certain actions with regard to the Guidelines after consideration by the RAC.

Some of the actions taken to date include the following:

1. The following experiment has been approved: The cloning in *B. subtilis,* under P2 conditions, of DNA derived from *Saccharomyces cerevisiae* using EK2 plasmid

*These follow the assigned containment levels as specified in the subsections of Section III–A with one exception. This exception is that experiments involving complete genomes of eukaryotic viruses will require P3+HV1 or P2+HV2 rather than the levels given in the subsections of Section III–A.

vectors provided that an HV1 *B. subtilis* host is used.

2. Unmodified laboratory strains of *Neurospora crassa* can be used in all experiments for which HV1 *N. crassa* systems are approved, provided that only DNA from Class 1 agents is used. For agents other than Class 1, unmodified laboratory strains of *N. crassa* can be used in all experiments for which HV1 *N. crassa* systems are approved, provided that these are carried out at physical containment one level higher than required for HV1. However, if P3 containment is specified for HV1 *N. crassa*, this level is considered adequate for unmodified *N. crassa*. Care must be exercised to prevent aerial dispersal of macroconidia, in accordance with good laboratory practice. Mutationally modified strains of *N. crassa* specified as HV1 in Appendix D can be used in all experiments for which HV2 *N. crassa* systems are approved, provided that only DNA from Class 1 agents is used.

3. P2 physical containment shall be used for DNA recombinants produced between members of the *Actinomycetes* group except for the species which are known to be pathogenic for man, animals, or plants.

4. Cloned desired fragments from any non-prohibited source may be transferred into *Agrobacterium tumefaciens* containing a Ti plasmid (or derivatives thereof), using a nonconjugative *E. coli* plasmid vector coupled to a fragment of the Ti plasmid and/or the origin of replication of an *Agrobacterium* plasmid, under containment conditions one step higher than would be required for the desired DNA in HV1 systems (i.e. one step higher physical containment than that specified in the subsections of Section III–A). However, DNA from plants and nonpathogenic prokaryotes may be cloned under P2 containment conditions; and the *Saccharomyces cerevisiae* alcohol dehydro-genase 1 gene and the gene coding for the maize (*Zea mays*) seed storage protein, zein, may be cloned under P1 conditions. Transfer into plant parts or cells in culture is permitted at the same containment level as is ued for the cloning in *Agrobacterium tumefaciens*.

5. *Bacillus subtilis* strains that do not carry an asporogenic mutation can be used as hosts specifically for the cloning of DNA derived from *E. coli* K–12 and *Streptomyces coelicolor, S. aureofaciens, S. rimosus, S. griseus, S. cyaneus,* and *s. venezuelae,* using NIH-approved *Staphylococcus aureau* plasmids as vectors under P2 conditions.

6. *Streptomyces coelicolor, S. auerofaciens, S. rimosus, S. griseus, S. cyaneus,* and *S. venezuelae* can be used as hosts for the cloning of DNA derived from *B. subtilis, E. coli,* K–12, or from *S. aureus* vectors that have been approved for use in *B. subtilis* under P2 conditions, using as vectors any plasmid indigenous to *Streptomyces* species or able to replicate in these hosts by natural biological mechanisms.

7. Certain cloned segments of *Anabena* DNA may be transferred into *Klebsiella* under P2 physical containment.

8. Permission is granted to clone foot-and-mouth disease virus in the EK1CV host-vector system consisting of *E. coli* K–12 and the vector pBR322, all work to be done at the Plumb Island Animal Disease Center.

9. Permission is granted to clone the Exotoxin A gene of *Pseudomonas aeruginosa* under P1 + EK1 conditions in *Escherichia coli* K–12 and under P1 conditions in *Pseudomonas aeruginosa*.

10. Permission is granted to return to the host of origin *Helminthosporanium maydis* (race O) DNA which has been cloned in yeast strain SHY2 using the hybrid *E. coli*—yeast plasmid Y1p5. The cloned DNA may be returned to, and propagated in, *Helminthosporanium maydis* at the P2 level of physical containment.

11. Permission is granted to return *Schizophyllum commune* DNA (or yeast DNA) cloned in *Saccharomyces cerevisiae* with YR or 2 mu circle vectors to *Schizophyllum commune*. The cloned DNA may be returned to, and propagated in, *Schizophyllum commune* at the P2 level of physical containment.

12. Permission is granted to return *Wangiella dermatitidis* DNA to *Wangiella dermatitidis* using an HV2 certified *Saccharomyces/E. coli* hybrid vector. The *Wangiella dermatitidis* may be propagated at the P3 level of physical containment.

13. Certain specified clones derived from segments of the Foot-and-Mouth Disease Virus may be transferred from Plum Island Animal Disease Center to the facilities of Genetech, Inc., of South San Francisco, California. Further development of the clones at Genentech has been approved under P1 + EK1 conditions.

14. *Saccharomycopsis lipolytica* may be used as a host for transformation with defined *Escherichia coli/Saccharomyces cerevisiae* hybrid plasmids and the hybrid plasmids may be used for cloning *S. lipolytica* DNA in *E. coli* and returning the cloned DNA to *S. lipolytica*.

15. Conjugative plasmids or transducing phages may be employed in recombinant DNA experiments when employing *E. coli* as host when a small defined segment of Adenovirus 2 DNA is employed as linker DNA.

16. Permission is granted to introduce DNA segments from aphid tramsmissible strains into non-aphid transmissible strains of Cauliflower mosiac virus in order to study the factors determining aphid transmissibility.

17. Permission is granted to return *Mucor racemosus* DNA which as been cloned in *Saccharomyces cerevisiae* host-vector systems to *Mucor racemosus*. In addition, permission is granted to transform *Mucor racemosus* with *S. cereviside* vectors with or without cloned *S. cerevisiae* sequences. These manipulations may be performed under P2 conditions.

18. DNA from nonpathogenic prokaryotes and nonpathogenic lower eukaryotes may be cloned into *Schizosaccharomyces pombe* species under P1 containment conditions. DNA from higher eukaryotes may be cloned in *S. pombe* species under P3 containment conditions.

19. The pyrogenic endotoxin type A (Tox A) gene of *Staphylococcus aureus* may be cloned in an HV2 *Bacillus subtilis* host-vector system under P3 containment conditions.

20. A hybrid plasmid composed of, (1) *E. coli* plasmid pBR325, (2) the origin of replication and transfer genes of *Agrobacterium tumefaciens* plasmid Ti, (3) the thiamine gene of *E. coli,* and (4) *Arabidopsis* DNA, may be transformed into *Agrobacterium tumefaciens* under P1 conditions. The *Agrobacterium tumefaciens* may subsequently be used to introduce the composite plasmid carrying *Arabidopsis* DNA and the *E. coli* thiamine gene into *Arabidopsis* plants under P1 containment conditions.

21. *Chlamydomonas reinhardi* can be used as a host for cloning defined DNA segments derived from *E. coli* and *Saccharomyces cerevisiae* using *E. coli/S. cerevisiae* hybrid vectors under P2 physical containment.

22. *Candida albicans* can be used as a host for cloning *Candida albicans* DNA following propagation of the DNA in *E. coli* K–12 or in *Saccharomyces cerevisiae* employing an *E. coli-S. cerevisiae* hybrid plasmid vector or the yeast 2 micron plasmid.

23. The Rd strain of *Hemophilus influenzae* can be used as a host for the propagation of the cloned Tn 10 tet R gene derived from *E. coli* K–12 employing the non-conjugative *Haemophilus* plasmid, pRSF0885, under P1 conditions.

24. *Zymomonas mobilis* may be used as a host under P2 conditions for transformation by recombinant DNA derived from *Pseudomonas* strains that are non-pathogenic for animals or plants, and that has been cloned in an *E. coli* K–12 host.

25. Protoplasts of *Streptosporangium brasiliense* may be transformed with a hybrid plasmid containing pBR322 plus a *Streptosporangium* plasmid into which have been incorporated specified DNA segments from *Streptomyces* species or an HV1 approved *Bacillus subtilis* cloning vector.

26. *Saccharomyces cerevisiae* DNA may be cloned in *Tetrahymena thermophila* using *E. coli/S. cerevisiae* hybrid plasmids under P1 containment conditions.

27. All members of the nonpathogenic *Actinomycetes* genus *Streptomyces* and the plasmids native to this genus are approved as host-vector systems for the cloning under P1 conditions of DNA derived from other nonpathogenic prokaryotic organisms such as *Streptomyces* and other nonpathogenic *Actinomycetes* species, *Escherichia coli* K–12, *Bacillus subtilis, Bacillus licheniformis, Bacillus circulans,* and other nonpathogenic *Bacillus* species, and for the cloning of DNA derived from nonpathogenic unicellular eukaryotic microorganisms such as *Saccharomyces cerevisiae* and *Neurospora crassa*.

28. *Bacillus subtilis* strains that do not carry an asporogenic mutation can be used under P2 conditions for the cloning of DNA from any CDC Class 1 organism, using indigenous plasmid and phage vectors whose host range does not include *Bacillus anthracis* or *Bacillus cereus*.

29. *Bacillus subtilis* strains that do not carry an asporogenic mutation can be used under P1 conditions for the cloning of DNA from any Class 1 *Bacillus* species, using indigenous plasmid and phage vectors whose host range does not include *Bacillus anthracis* or *Bacillus cereus*.

30. Permission is granted to clone in *E. coli* K–12, in high containment Building 550 at the Frederick Cancer Research Center, restriction fragments of *Corynephage Beta* carrying the structural gene for diphtheria toxin. Laboratory practices and containment equipment are to be specified by the IBC.

31. Permission is granted to clone certain subgenomic segments of Foot and Mouth Disease Virus in HV1 *Bacillus subtilis* and *Saccharomyces cerevisiae* host-vector systems under P1 conditions at Genentech, Inc., South San Francisco, California.

32. Permission is granted in principle to propagate in mammalian cell culture recombinant DNA molecules consisting of segments of Foot and Mouth Disease Virus and SV40 deletion vectors under P3 conditions at the Plum Island Animal Disease Center. Approval is subject to review by a RAC Working Group of individual experiments.

33. A conjugative plasmid may be used to transfer among *E. coli* K–12 strains, under P2 physical containment, the qa–2 of *Neurospora crassa* ligated to a mobilizable plasmid.

34. *E. coli* K–12 strain DF214 (or derivatives thereof) and plasmid vectors (e.g., pBR322, pBR325) may be used to clone rat cDNA under P2 conditions. After the clone of interest has been purified, it may be worked with under P1 containment.

35. Permission is granted to Dr. Ronald Davis of Stanford University to field test corn plants modified by recombinant DNA techniques under specified containment conditions.

36. Permission is granted to clone in *E. coli* K–12, under P1 physical containment conditions, subgenomic segments of Rift Valley Fever Virus Subject to conditions which have been set forth by the RAC.

37. Permission is given to transfer a recombinant lactose plasmid from *Streptococcus faecalis* to *S. lactis* by conjugation.

38. Attenuated laboratory strains of *Salmonella typhimurium* may be used under P1 physical containment conditions to screen for the *Saccharomyces cerevisiae* pseudouridine synthetase gene. The plasmid YEp13 will be employed as the vector.

39. Permission is granted to clone in *Haemophilus parainfluenzae* Moloney murine leukemia provirus and mouse cellular flanking sequences employing the plasmid vector, pRK290, under P2 containment conditions.

40. Permission is granted for the development, under P1 conditions, of a new host-vector system based on the use of *Corynebacterium glutamicum* as host and non-conjugative poorly mobilizable plasmids as vectors.

41. *Vibrio harveyi* DNA may be cloned in *Vibrio cholera*; plasmids may be used to transfer the cloned *V. harveyi* DNA between *E. coli* K–12, *V. cholera*, and *V. harveyi*. P2 physical containment conditions are required for those experiments involving *V. cholera*. P1 containment conditions may be used for other phases of the project.

Appendix F of Annex E.—Certified Host-Vector Systems

While many experiments using *E. coli* K–12, *Saccharomyces cerevisiae* and *Bacillus subtilis* are currently exempt from the Guidelines under Exemption I–E–5, some derivatives of these host-vector systems were previously classified as HV1 or HV2. A listing of those systems follows.

HV1—The following plasmids are accepted as the vector components of certified *B. subtilis* HV1 systems: pUB110, pC194, pS194, pSA2100, pE194, pT127, pUB112, pC221, pC223, and pAB124. *B. subtilis* strains RUB 331 and BGSC 1553 have been certified as the host component of HV1 systems based on these plasmids.

HV2—The asporogenic mutant derivative of *Bacillus subtilis*, ASB 298, with the following plasmids as the vector component: pUB110, pC194, pS194, pSA2100, pE194, pT127, pUB112, pC221, pC223, and pAB124.

HV2—The following sterile strains of *Saccharomyces cerevisiae*, all of which have the ste–VC9 mutation, SHY1, SHY2, SHY3, and SHY4. The following plasmids are certified for use: YIp1, YEp2, YEp4, YIp5, YEp6, YRp7, YEp20, YEp21, YEp24, YIp25, YIp26, YIp27, YIp28, YIp29, YIp30, YIp31, YIp32 and YIp33.

EK2 Plasmid Systems. The *E. coli* K–12 strain chi–1776. The following plasmids are certified for use: pSC101, pMB9, pBR313, pBR322, pDH24, pBR327, pGL101, pHB1. The following *E. coli/S. cerevisiae* hybrid plasmids are certified as EK2 vectors when used in *E. coli* chi–1776 or in the sterile yeast strains, SHY1, SHY2, SHY3 and SHY4: YIp1, YEp2, YEp4, YIp5, YEp6, YRp7, YEp20, YEp21, YEp24, YIp25, YIp26, YIp27, YIp28, YIp29, YIp30, YIp31, YIp32, YIp33.

EK2 Bacteriophage Systems: The following are certified EK2 systems based on bacteriophage lambda.

Vector	*Host*
λgt*WES*. λB′	DP50*supF*
λgt*WES*. λB*	DP50*supF*
λgtZJ*vir*. λB′	*E. coli* K–12
λgtALO. λB	DP50*supF*
Charon 3A	DP50 or DP50*supF*
Charon 4A	DP50 or DP50*supF*
Charon 16A	DP50 or DP50*supF*
Charon 21A	DP50*supF*
Charon 23A	DP50 or DP50*supF*
Charon 24A	DP50 or DP50*supF*

Appendix G of Annex E.—Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Toxins for Vertebrates

1. General Information

Appendix G specifies the containment to be used for the deliberate cloning of genes coding for the biosynthesis of toxins for vertebrates. Cloning of genes coding for toxins for vertebrates that have an LD_{50} of less than 100 nanograms per kilogram body weight (e.g., the botulinum toxins, tetanus toxin, diphtheria toxin, *Shigella dysenteriae* neurotoxin) is prohibited. No specific restrictions shall apply to the cloning of genes if the protein specified by the gene has an LD_{50} of 100 micrograms or more per kilogram of body weight. Experiments involving genes coding for toxins with an LD_{50} of 100 micrograms or less per kilogram body weight shall be registered with ORDA prior to initiating the experiments. A list of toxins classified as to LD_{50} is available from ORDA. Testing procedures for determining toxicity of toxins not on the list are available from ORDA. The results of such tests shall be forwarded to ORDA, which will consult with the *ad hoc* Working Group on toxins prior to inclusion of the toxin on the list. (See Section IV–E–1–b–(3)–(i).)

2. Containment Conditions for Cloning of Toxin Genes in E. coli K–12

(a) Cloning of genes coding for toxins for vertebrates that have an LD_{50} in the range of 100 nanograms to 1000 nanograms per kilogram body weight (e.g., abrin, *Clostridium perfringens* epsilon toxin) may proceed under P2 + EK2 or P3 + EK1 containment conditions.

(b) Cloning of genes for the biosynthesis of toxins for vertebrates with an LD_{50} in the range of 1 microgram to 100 micrograms per kilogram body weight may proceed under P1 + EK1 containment conditions (e.g., *Staphylococcus aureus* alpha toxin, *Staphylococcus aureus* beta toxin, ricin, *Pseudomonas aeruginosa* exotoxin A, *Bordatella pertussis* toxin, the lethal factor of *Bacillus anthracis*, the *Pasteurella pestis* murine toxins, the oxygen-labile hemolysins such as streptolysin O, and certain neurotoxins present in snake venoms and other venoms).

(c) Some enterotoxins are substantially more toxic when administered internally than parenterally. The following enterotoxins shall be subject to P1 + EK1 containment conditions: cholera toxin, the heat labile toxins of *E. coli*, *Klebsiella*, and other related proteins that may be identified by neutralization with an antiserum mono-specific for cholera toxin, and the heat stable toxins of *E. coli* and of *Yersinia enterocolitica*.

3. Containment Conditions for Cloning of Toxins Genes in Organisms Other Than E. coli K-12

Requests involving the cloning of genes coding for toxins for vertebrates in host-vector systems other than *E. coli* K-12 will be evaluated by ORDA, which will consult with the *ad hoc* working group on toxins. (See Section IV-E-1-b-(3)-(j).)

Appendix H of Annex E.—Experiments Covered by Section III-0

No experiments currently fall under Section III-0 of the Guidelines.

Note.—OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the *Catalog of Federal Domestic Assistance*. Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every federal program would be included as many federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the *Catalog of Federal Domestic Assistance* are affected.

NIH programs are not covered by OMB Circular A-95 because they fit the description of "programs not considered appropriate" in Section 8-(b)-(4) and (5) of that Circular.

Dated: October 26, 1981.

Richard M. Krause,

Director, National Institute of Allergy and Infectious Diseases.

[FR Doc. 81-34469 Filed 12-3-81; 8:45 am]

BILLING CODE 4110-08-M

federal register

Monday
December 7, 1981

Part II

Department of Health and Human Services

National Institutes of Health

Recombinant DNA Research; Proposed Actions Under Guidelines

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Recombinant DNA Research; Proposed Actions Under Guidelines

AGENCY: National Institutes of Health, PHS, DHHS.

ACTION: Notice to Proposed Actions under NIH Guidelines for Research Involving Recombinant DNA Molecules.

SUMMARY: This notice sets forth proposed actions to be taken under the NIH Guidelines for Research Involving Recombinant DNA Molecules. Interested parties are invited to submit comments concerning these proposals. After consideration of these proposals and comments by the NIH Recombinant DNA Advisory Committee (RAC) at its next meeting, the Director of the National Institute of Allergy and Infectious Diseases will issue decisions on these proposals in accord with the Guidelines.

DATE: Comments must be received by February 1, 1982.

ADDRESS: Written comments and recommendations should be submitted to the Director, Office of Recombinant DNA Activities, Building 31, Room 4A52, National Institutes of Health, Bethesda, Maryland, 20205. All comments received in timely response to this notice will be considered and will be available for public inspection in the above office on weekdays between the hours of 8:30 a.m. and 5:00 p.m.

FOR FURTHER INFORMATION CONTACT: Background documentation and additional information can be obtained from Drs. Stanley Barban or Elizabeth Milewski, Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland, 20205, (301) 496-6051.

SUPPLEMENTARY INFORMATION: The National Institutes of Health will consider the following changes and amendments under the Guidelines for Research Involving Recombinant DNA Molecules, as well as actions under these Guidelines.

1. Proposed Revision of Guidelines

Dr. Waclaw Szybalski, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin, has requested that the following sentence be added to the current NIH Guidelines:

"These are only Guidelines based on the current state of the research and on the concerns of certain individuals or groups about the hypothetical risks. Individual researchers should use their best scientific judgement and peers' advice when designing, modifying and conducting experiments, and be prepared to justify any substantial variations when requested, or in their applications and progress reports."

2. Request To Use Bacillus Megaterium in Recombinant DNA Experiments Under P1 Containment

Dr. Patricia Vary of Northern Illinois University, DeKalb, Illinois, requests permission to introduce recombinant DNA derived from *Staphylococcus aureus, E. coli,* and *Bacillus subtilis* into *Bacillus megaterium* under P1 conditions.

3. Proposed EK2 Host-Vector Systems

Dr. Roy Curtiss, University of Alabama, Birmingham, Alabama, requests EK2 certification of six different *E. coli* K-12 strains in conjunction with various virulent and temperate bacteriophage lambda, plasmid and cosmid vectors. Dr. Curtiss also requests that all previously approved vectors be approved as vector components of the proposed EK2 host-vector systems. Detailed information on the proposed host-vector systems has been provided by Dr. Curtiss.

4. Proposed use of EK2 Host-Vector Systems for Cloning DNA from Class 3 and 4 Etiologic Agents

Dr. Roy Curtiss, University of Alabama, Birmingham, Alabama, requests permission to use all certified EK2 host-vector systems to clone DNA fragments from Class 3 and Class 4 etiologic agents under P2 containment, and under P1 containment if the recombinant clones are shown not to express a virulence determinant that has toxic potential.

As an alternative to this general proposal, Dr. Curtiss requests permission to clone DNA from *Yersinia pestis* and *Mycobacterium leprae* into EK2 host-vector systems under P2 containment, and under P1 conditions in the absence of expression of virulence determinants by the recombinant clones.

5. Proposed Pseudomonas Putida Host-Vector System

Dr. Michael Bagdasarian of the Max-Planck Institute of Fur Molekulare Genetik, Berlin, West Germany, requests HV1 certification of a host-vector system based on *Pseudomonas putida* strain KT2440 and cloning vectors pKT262, pKT263, and pKT264.

6. Proposed Inclusion of Yersinia Enterocolitica on Sublist A, Appendix A

Dr. Guy Cornelis of the Universite Catholique de Louvain, Brussels, Belgium, requests that *Yersinia enterocolitica* be exempted from the Guidelines under Section I-E-4 and added to Sublist A, Appendix A, on the basis that this species exchanges genetic information with *E. coli.*

7. Proposal for Revision of the Guidelines

Dr. Susan Gottesman of the National Cancer Institute of the National Institutes of Health has requested that the following proposal to modify the NIH Guidelines for Research Involving Recombinant DNA Molecules be published in the **Federal Register** for comment:

I. Introduction

There seems to be a clear consensus that the guidelines require simplification, reorganization, and possible lowering of containment levels for some classes of experiments. The Recombinant DNA Advisory Committee (RAC) will be considering at its next meeting a proposal, published for comment in the **Federal Register,** to convert the NIH Recombinant DNA Guidelines into a voluntary code of practice. This proposal would change the detailed listing of containment levels to a few paragraphs of advice to the investigator. I am recommending an alternative approach to revision which retains some aspects of the current guidelines which I consider necessary for the continued orderly development of the recombinant DNA field.

(1) My proposal, as opposed to the RAC proposal, retains the requirement for IBC oversight of some experiments, and retains the current NIH compliance requirements. In addition, the record-keeping function of the IBC is retained. Since many experiments became exempt from the requirements of the Guidelines as of July 1, 1981, the remaining requirements should not be too cumbersome to either the experimenter or the IBC.

(2) My proposal simplifies the structure of the Guidelines and decreases containment for some classes of experiments. In particular, experiments involving non-pathogenic prokaryotes and lower eukaryotes could be carried out at P1 containment. Overall, containment levels would not, however, be lowered to the extent recommended in the RAC proposal.

II. Summary of Proposed Changes as Compared to Current Guidelines

A. *Prohibitions.* The word "prohibition" will no longer be applied to this class of experiments which

currently require explicit RAC review and NIH approval.

1. Three of the five prohibitions (I–D–2, I–D–4, and I–D–5 in the current Guidelines) would be listed in a new section designated III–A and entitled "Experiments that require FAC review and NIH approval before initiation." These three prohibitions cover experiments involving the deliberate formation of recombinant DNAs containing genes for the biosynthesis of certain toxins lethal for vertebrates (I–D–2), the deliberate release into the environment of organisms containing recombinant DNA (I–D–4), and the 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 a drug to control disease agents in human or veterinary medicine or agriculture (I–D–5).

2. Prohibition I–D–1 in the current guidelines (formation of recombinant DNAs derived from CDC Class 4 or 5 organisms) would be deleted. Such experiments could be carried out under specific conditions set by the IBC, as described in a new section designated III–B and entitled "Experiments that require IBC approval before initiation".

3. Prohibition I–D–6 in the current Guidelines, which covers certain large-scale experiments, would be eliminated; such experiments could proceed after IBC approval (new section III–B–5). Currently the Guidelines permit certain large scale experiments involving *E. coli* K–12, *S. cerevisiae* and *B. subtilis* host-vector systems to proceed following review by the local IBC (46 FR 53983).

B. *Exemptions.* No changes in the substance of this section are proposed. The categories of exemptions now listed in Section I–E would be listed in a new Section III–D.

C. *Section II (Containment).* All references to HV3 systems would be deleted. Such systems are not presently in use and are not required by current or proposed guidelines. Otherwise, the section would remain as is.

D. *Section III (Containment Guidelines for Covered Experiments).* The complete text of Section III would be replaced by new text which would reorganize, simplify, and eliminate redundancy in the current Guidelines. In addition, significant lowering of containment for some classes of experiments is recommended. In the new Section III, all experiments would fall into one of four classes:

III–A. Current prohibitions; such experiments would require specific RAC review and NIH approval.

III–B. Experiments requiring prior review by the IBC. These would include the use of recombinant DNA molecules derived from or propagated in pathogens, non-defective animal virus systems, and the insertion of recombinant DNA into whole animals and plants. Many experiments which would be included in this section currently require NIH approval.

III–C. Experiments requiring notice to the IBC simultaneously with initiation of the experiment.

The proposal would change the procedure for reviewing recombinant DNA experiments involving non-pathogenic prokaryotes, lower eukaryotes, or defective animal virus systems. These experiments currently require prior review by the IBC. Under this proposal, investigators would not have to wait for IBC approval before initiating these experiments. In addition, containment requirements for experiments involving non-pathogenic prokaryotes and non-pathogenic lower eukaryotes would be relaxed.

III–D. Currently exempt experiments. No changes in the substance of this section are proposed.

E. Section IV. Changes as necessary to reflect changes in other sections of the Guidelines would be incorporated.

F. Much material currently in Appendix E would be covered or superseded by the proposed changes in the Guidelines in Section III. Appendix D would be deleted.

III. Changes in Current Guidelines to Implement Proposal

A. *Current Section I.* Section I–A, *Purpose,* would remain unchanged.

Section I–B, *Definition of Recombinant DNA Molecules,* would be modified to include a statement on synthetic DNAs, and would read as follows:

"I–B. *Definition of Recombinant DNA Molecules.* In the context of these Guidelines, recombinant DNA molecules are defined as either (i) 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 (i) 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 polynucleotide or polypeptide product, it is exempt from the Guidelines."

Section I–C, *General Applicability,* would remain unchanged.

Section I–D, *Prohibitions,* would be deleted. (Some of this material is included in proposed new Section III–A).

Section I–E, *Exemptions,* would be deleted. (This material would be included in proposed new Section III–D).

B. *Current Section II.* Delete current Section II–D–1–c and subsections II–D–1–c–(1) through II–D–1–c–(5), Section II–D–2–b–(3) and references to HV3 in Section II–D–3.

C. *Current Section III.* Delete and replace current Section III and all subsections of III with the following text:

"Part III discusses experiments involving recombinant DNA. These experiments have been divided into four classes:

"III–A. Experiments which require specific RAC review and NIH approval before initiation of the experiment;

"III–B. Experiments which require IBC approval before initiation of the experiment;

"III–C. Experiments which require IBC notification at the time of initiation of the experiment;

"III–D. Experiments which are exempt from the procedures of the Guidelines.

"If an experiment falls into both class III–A and one of the other classes, the rules pertaining to class III–A must be followed. If an experiment falls into class III–D and into either class III–B or III–C as well, it can be considered exempt from the requirements of the Guidelines.

"Changes in containment levels from those specified here may not be instituted without the express approval of the Director, NIH (See Sections IV–E–1–b–(1), IV–E–1–b–(2), and subsections).

"III–A. *Experiments that Require RAC Review and NIH Approval Before Initiation.* Experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH, the publication of the proposal in the **Federal Register** for thirty days of comment, review by the RAC, and specific approval by NIH. The containment conditions for such experiments will be recommended by RAC and set by NIH at the time of approval. Such experiments also require the approval of the IBC before initiation. Specific experiments already approved in this section and the appropriate containment conditions are listed in Appendices E and G.

"III–A–1. Deliberate formation of recombinant DNAs containing genes for the biosynthesis of toxins lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight (e.g., the botulinum toxins, tetanus toxin, diphtheria toxin, *Shigella dysenteriae*

neurotoxin). Specific approval has been given for the cloning in *E. coli* K-12 of DNAs containing genes coding for the biosynthesis of toxins which are lethal to vertebrates at 100 nanograms to 100 micrograms per kilogram body weight. Containment levels for these experiments are specified in Appendix G.

"III-A-2. Deliberate release into the environment of any organism containing recombinant DNA.

"III-A-3. Deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire it naturally, if such acquistion could compromise the use of the drug to control disease agents in human or veterinary medicine or agriculture.

"III-B. *Experiments that Require IBC Approval Before Initiation.* Investigators performing experiments in this category must submit to their Institutional Biosafety Committee (IBC), prior to initiation of the experiments, a registration document that contains a description of: (a) The source(s) of DNA, (b) the nature of the inserted DNA sequences, (c) the hosts and vectors to be used, (d) whether a deliberate attempt will be made to obtain expression of a foreign gene, and, if so, what protein will be produced, and (e) the containment conditions specified in these Guidelines. This registration document 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 NIH (See Section IV-E-1-b-(3)).

"III-B-1. *Experiments Using CDC Class 2, Class 3, Class 4, or Class 5 Agents* as Host-Vector Systems.*

"III-B-1-a. Experiments involving the introduction of recombinant DNA into CDC Class 2 agents can be carried out at P2 containment.

"III-B-1-b. Experiments involving the introduction of recombinant DNA into CDC Class 3 agents can be carried out at P3 containment.

"III-B-1-c. Experiments involving the introduction of recombinant DNA into CDC Class 4** or Class 5 agents can be carried out at P4 containment. A USDA permit is required for work with Class 5 agents***.

"III-B-2. *Experiments in Which DNA from CDC Class 2 or Class 3 Agents* is Cloned in Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems.*

"III-B-2-a. Recombinant DNA experiments in which DNA from CDC Class 2 or Class 3 agents* is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under P2 containment. Specific lowering of containment to P1 for particular experiments can be approved by the IBC. Many experiments in this category will be exempt from the Guidelines (See Sections III-D-4 and III-D-5). Experiments involving the formation of recombinant DNAs for certain toxin genes requires RAC review and NIH approval (see Section III-A-1), or must be carried out under NIH specified conditions as described in Appendix G.

"III-B-2-b. Recombinant DNA experiments in which DNA from CDC Class 4** or Class 5 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes can be performed at P2 containment after demonstration that only a totally and irreversibly defective fraction of the agent's viral genome is present in a given recombinant. In the absence of such a demonstration, P4 containment should be used.

Note.—A USDA permit is required for work with Class 5 pathogens***.

"III-B-3. *Experiments Involving the Use of Whole Animal or Plant Viruses or Defective Animal or Plant Viruses in the Presence of Helper Virus in Tissue Culture Systems.*

"III-B-3-a. Experiments involving the use of whole CDC Class 2 animal viruses*, or defective CDC Class 2 animal viruses* in the presence of helper virus, can be performed at P2 containment.

"III-B-3-b. Experiments involving the use of whole CDC Class 3 animal viruses*, or defective CDC Class 3 animal viruses* in the presence of helper virus, can be carried out at P3 containment.

"III-B-3-c. Experiments involving the use of whole CDC Class 4 or Class 5 animal viruses*, or defective CDC Class 4 or Class 5 animal viruses* in the presence of helper virus, may be carried out under P4 containment. A USDA permit is required for work with CDC Class 5 pathogens***.

"III-B-3-d. Experiments involving the use of whole animal or plant viruses, or defective animal or plant viruses in the presence of helper virus, not covered by Sections III-B-3-a, III-B-3-b, or III-B-3-c may be carried out under P1 containment.

"III-B-4. *Recombinant DNA Experiments Involving Whole Animals and Plants.*

"III-B-4-a. DNA from any source except for greater than one quarter of a eukaryotic viral genome may be transferred to any non-human vertebrate organism and propagated under conditions of physical containment comparable to P1 and appropriate to the organism under study (2A).

"III-B-4-b. For all experiments involving whole animals and plants and not covered by III-B-4-a, the appropriate containment will be determined by the IBC.

"III-B-5. *Experiments Involving More than 10 Liters of Culture.*

The appropriate containment will be decided by the IBC. Where appropriate, the large scale containment recommendations of the NIH should be used (45 FR 24968).

"III-C. *Experiments that Require IBC Notice Simultaneously with Initiation of Experiments.*

"For experiments in this category, a registration document as described in Section III-B must be dated and signed by the investigator and filed with the local IBC. The IBC shall review all such proposals, but IBC review prior to initiation of the experiment is not required. All experiments not included in categories III-A, III-B, III-D, and subsections of these categories are to be considered as in category III-C. All such experiments can be carried out at P1 containment. Some explicit cases are listed below.

"III-C-1. *Experiments Involving Non-Pathogenic Prokaryotes or Non-Pathogenic Lower Eukaryotic Host-Vector Systems.*

"Experiments involving non-pathogenic prokaryotes or non-pathogenic lower eukaryotes can be carried out at P1 containment.

"III-C-2. *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 (36) being considered identical (50)) may be propagated and maintained in cells in tissue culture using P1 containment. For such experiments, it must be shown that the cells lack helper virus for the specific Families of defective viruses being 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.

"III-D. *Exempt Experiments.*

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

"III-D-1. Those that are not in organisms or viruses.

"III-D-2. Those that consist entirely of DNA segments from a single non-

chromosomal or viral DNA source, though one or more of the segments may be synthetic equivalent.

"III–D–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 a 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).

"III–D–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. A list of such exchangers will be prepared and periodically revised by the Director, NIH, with advice of the RAC, after appropriate notice and opportunity for public comment. (See Section IV–E–1–b–(1)–(d).) Certain classes are exempt as of publication of these Revised Guidelines. The list is in Appendix A. An updated list may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20205.

"III–D–5. Other classes of recombinant DNA molecules, if the Director, NIH, 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. (See Section IV–E–1–b–(1)–(d).) Certain classes are exempt as of publication of these Revised Guidelines. The list is in Appendix C. An updated list may be obtained from the Office of Recombinant DNA Activities; National Institutes of Health, Bethesda, Maryland 20205."

D. *Current Section IV. Roles and Responsibilities.* Changes as necessary to reflect changes in Parts I–III only.

E. *Current Section V. Footnotes and References.* Changes as necessary to reflect changes in Parts I–III.

F. *Current Appendices.* Delete sections subsumed by proposed guideline changes; i.e. delete Appendix D and entries 1, 2, 3, 5, 7, 10, 11, 12, 14, 15, 17, 18, 20, 22, 24, 25, 26, 27, 28, 29, 33, and 34 of Appendix E.

Footnotes

* *Classification of Etiologic Agents on the basis of Hazard.* (4th Edition, July 1974). U.S. Department of Health, Education and Welfare, Public Health Service, Centers for Disease Control, Office of Biosafety, Atlanta, Georgia 30333.

** All activities, including storage of variola and whitepox are retricted to the single national facility (World Health Organization (WHO) Collaborating Center for Smallpox Research, Center for Disease Control, in Atlanta).

*** A USDA permit, required for import and interstate commerce of pathogens, may be obtained from the Animal and Plant Health Inspection Service USDA, Federal Building, Hyattsville, MD 20782.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the *Catalog of Federal Domestic Assistance.* Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require serveral additional pages. In addition, NIH could not be certain that every federal program would be included as many federal agencies, as well as private organizations both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the *Catalog of Federal Domestic Assistance* are affected.

NIH programs are not covered by OMB Circular A–95 because they fit the description of "programs not considered appropriate" in Section 8–(b)–(4) and (5) of that Circular.

Dated: November 25, 1981.

Richard M. Krause,

Director, National Institute of Allergy and Infectious Diseases, National Institutes of Health.

[FR Doc. 81–34468 Filed 12–4–81; 8:45 am]

BILLING CODE 4140–01–M

4. S-16

Comm. from William J. Gartland, Jr., Dir., Office of Recombinant DNA Activities, Dept. of Health and Human Services Re: proposed revisions of NIH Guidelines for Recombinant DNA research.

Copy sent to City Manager and Comm. of Health and Hospitals 1/12/82 mh

In City Council,

January 11, 1982

1/11/82

Referred to the City Manager and Commissioner of Health and Hospitals