For the Commission, by the Division of Market Regulation, pursuant to delegated authority, 17 CFR 200.30–3(a)(12).

Jonathan G. Katz,

Secretary.

[FR Doc. 94–16187 Filed 7–1–94; 8:45 am]

BILLING CODE 8010–01–M

Self-Regulatory Organization; Applications for Unlisted Trading Privileges; Notice and Opportunity for Hearing; Chicago Stock Exchange, Inc.

June 27, 1994.

The above named national securities exchange has filed applications with the Securities and Exchange Commission ("Commission") pursuant to Section 12(f)(1)(B) of the Securities Exchange Act of 1934 and Rule 12f–1 thereunder for unlisted trading privileges in the following securities:

Jalate, Ltd.
Common Stock, No Par Value (File No. 7–12589)
Alliances Entertainment Corp.
Warrants A 5/13/95 (File No. 7–12590)
Alliances Entertainment Corp.
Warrants B 5/13/95 (File No. 7–12591)
Energy Ventures, Inc.
Common Stock, $1.00 Par Value (File No. 7–12592)
Highwoods Properties, Inc.
Common Stock, $.01 Par Value (File No. 7–12593)
International Lottery, Inc.
Common Stock, $.01 Par Value (File No. 7–12594)
Liberty Property Trust
Shares of Beneficial Interest, $.001 Par Value (File No. 7–12595)
Watsco, Inc.
Common Stock, $.50 Par Value (File No. 7–12596)

These securities are listed and registered on one or more other national securities exchanges and are reported in the consolidated transaction reporting system.

Interested persons are invited to submit on or before July 19, 1994, written data, views and arguments concerning the above-referenced application. Persons desiring to make written comments should file three copies thereof with the Secretary of the Securities and Exchange Commission, 450 Fifth Street, NW., Washington, DC 20549. Following this opportunity for hearing, the Commission will approve the application if it finds, based upon all the information available to it, that the extensions of unlisted trading privileges pursuant to such application is consistent with the maintenance of fair and orderly markets and the protection of investors.

For the Commission, by the Division of Market Regulation, pursuant to delegated authority.

Jonathan G. Katz,

Secretary.

[FR Doc. 94–16120 Filed 7–1–94; 8:45 am]

BILLING CODE 8010–01–M

DEPARTMENT OF THE TREASURY

Debt Management Advisory Committee; Meeting

Notice is hereby given, pursuant to 5 U.S.C. App. 10(a)(2), that a meeting will be held at the U.S. Treasury Department, 15th and Pennsylvania Avenue, NW., Washington, DC, on August 2 and 3, 1994, of the following debt management advisory committee:

Public Securities Association, Treasury Borrowing Advisory Committee

The agenda for the meeting provides for a technical background briefing by Treasury staff on August 2, followed by a charge by the Secretary of the Treasury or his designate that the committee discuss particular issues, and a working session. On August 3, the committee will present a written report of its recommendations.

The background briefing by Treasury staff will be held at 11:30 a.m. Eastern time on August 2 and will be open to the public. The remaining sessions on August 2 and the committee's reporting session on August 3 will be closed to the public, pursuant to 5 U.S.C. App. 10(d).

This notice shall constitute my determination, pursuant to the authority placed in heads of departments by 5 U.S.C. App. 10(d) and vested in me by Treasury Department Order No. 101–05, that the closed portions of the meeting are concerned with information that is exempt from disclosure under 5 U.S.C. 552b(c)(9)(A). The public interest requires that such meetings be closed to the public because the Treasury Department requires frank and full advice from representatives of the financial community prior to making its final decision on major financing operations. Historically, this advice has been offered by debt management advisory committees established by the several major segments of the financial community. When so utilized, such a committee is recognized to be an advisory committee under 5 U.S.C. App. 3.

Although the Treasury's final announcement of financing plans may not reflect the recommendations provided in reports of the advisory committee, premature disclosure of the committee's deliberations and reports would be likely to lead to significant financial speculation in the securities market. Thus, these meetings fall within the exemption covered by U.S.C. 552b(c)(9)(A).

The Office of the Under Secretary for Domestic Finance is responsible for maintaining records of debt management advisory committee meetings and for providing annual reports setting forth a summary of committee activities and such other matters as may be informative to the public consistent with the policy of 5 U.S.C. 552b.

Dated: June 28, 1994.

Frank N. Newman,

Under Secretary of the Treasury, Domestic Finance.

[FR Doc. 94–16154 Filed 7–1–94; 8:45 am]

BILLING CODE 4810–25–M

Fiscal Service

Renegotiation Board Interest Rate, Prompt Payment Interest Rate, Contracts Disputes Act

Although the Renegotiation Board is no longer in existence, other Federal Agencies are required to use interest rates computer under the criteria established by the Renegotiation Act of 1971 (P.L. 92–41). For example, the Contracts Disputes Act of 1978 (P.L. 95–563) and the Prompt Payment Act (P.L. 97–177) are required to calculate interest due on claims at a rate established by the Secretary of the Treasury pursuant to Public Law 92–41 (85 Stat. 97) for the Renegotiation Board (31 U.S.C. 3902).

Therefore, notice is hereby given that, pursuant to the above mentioned sections, the Secretary of the Treasury has determined that the rate of interest applicable for the purpose of said sections, for the period beginning July 1, 1994 and ending on December 31, 1994, is 7% per centum per annum.

Dated: June 28, 1994.

Marcus W. Page,

Acting Fiscal Assistant Secretary.

[FR Doc. 94–16116 Filed 7–1–94; 8:45 am]

BILLING CODE 4810–35–M

Sunshine Act Meetings

Federal Register
Vol. 59, No. 127
Tuesday, July 5, 1994

This section of the FEDERAL REGISTER contains notices of meetings published under the "Government in the Sunshine Act" (Pub. L. 94-409) 5 U.S.C. 552b(e)(3).

BOARD OF GOVERNORS OF THE FEDERAL RESERVE SYSTEM

TIME AND DATE: 10 a.m., Thursday, July 7, 1994.

PLACE: Marriner S. Eccles Federal Reserve Board Building, C Street entrance between 20th and 21st Streets, NW., Washington, DC 20551.

STATUS: Open.

MATTERS TO BE CONSIDERED:

1. Publication for comment of a draft policy statement containing standards for privately operated large-dollar multilateral netting systems.
2. Proposed 1995 Federal Reserve Board budget objective.
3. Any items carried forward from a previously announced meeting.

Note: This meeting will be recorded for the benefit of those unable to attend. Cassettes will be available for listening in the Board's Freedom of Information Office, and copies may be ordered for $5 per cassette by calling (202) 452–3684 or by writing to: Freedom of Information Office, Board of Governors of the Federal Reserve System, Washington, DC 20551.

CONTACT PERSON FOR MORE INFORMATION: Mr. Joseph R. Coyne, Assistant to the Board; (202) 452–3204.

Dated: June 30, 1994.

Jennifer J. Johnson,

Associate Secretary of the Board.

[FR Doc. 94–16307 Filed 6–30–94; 3:03 pm]

BILLING CODE 6210–01–P

BOARD OF GOVERNORS OF THE FEDERAL RESERVE SYSTEM

TIME AND DATE: Approximately 11:00 a.m., Thursday, July 7, 1994.

PLACE: Marriner S. Eccles Federal Reserve Board Building, C Street entrance between 20th and 21st Streets, N.W., Washington, D.C. 20551.

STATUS: Closed.

MATTERS TO BE CONSIDERED:

1. Personnel actions (appointments, promotions, assignments, reassignments, and salary actions) involving individual Federal Reserve System employees.
2. Any items carried forward from a previously announced meeting.

CONTACT PERSON FOR MORE INFORMATION: Mr. Joseph R. Coyne, Assistant to the Board; (202) 452–3204. You may call (202) 452–3207, beginning at approximately 5 p.m. two business days before this meeting, for a recorded announcement of bank and bank holding company applications scheduled for the meeting.

Dated: June 30, 1994.

Jennifer J. Johnson,

Associate Secretary of the Board.

[FR Doc. 94–16308 Filed 6–30–94; 3:03 pm]

BILLING CODE 6210–01–P

SECURITIES AND EXCHANGE COMMISSION

Agency Meeting

Notice is hereby given, pursuant to the provisions of the Government in the Sunshine Act, Pub. L. 94–409, that the Securities and Exchange Commission will hold the following meeting during the week of July 4, 1994.

A closed meeting will be held on Thursday, July 7, 1994, at 10 a.m.

Commissioners, Counsel of the Commissioners, the Secretary to the Commission, and recording secretaries will attend the closed meeting. Certain staff members who have an interest in the matters may also be present.

The General Counsel of the Commission, or his designee, has certified that, in his opinion, one or more of the exemptions set forth in 5 U.S.C. 552b(c) (4), (8), (9)(A) and (10) and 17 CFR 200.402(a) (4), (8), (9)(i) and (10), permit consideration of the scheduled matters at a closed meeting.

Commissioner Roberts, as duty officer, voted to consider the items listed for the closed meeting in the closed session.

The subject matter of the closed meeting scheduled for Thursday, July 7, 1994, at 10 a.m., will be:

Institution of administrative proceedings of an enforcement nature.
Settlement of administrative proceedings of an enforcement nature.
Institution of injunctive actions.
Settlement of injunctive actions.

At times, changes in Commission priorities require alterations in the scheduling of meeting items. For further information and to ascertain what, if any, matters have been added, deleted or postponed, please contact: Paul Atkins (202) 942–0100.

Dated: June 29, 1994.

Jonathan G. Katz,

Secretary.

[FR Doc. 94–16268 Filed 6–30–94; 11:44 am]

BILLING CODE 8010–01–M

Federal Register

Tuesday
July 5, 1994

Part II

Environmental Protection Agency

Sole Source Aquifer Designation of the Vashon-Maury Island Aquifer System, King County, Washington; Notice

ENVIRONMENTAL PROTECTION AGENCY

[FRL–5006–8]

Sole Source Aquifer Designation of the Vashon-Maury Island Aquifer System, King County, Washington

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final determination.

SUMMARY: The Region 10 Administrator of the Environmental Protection Agency (EPA) has determined that the Vashon-Maury Island Aquifer System is a sole or principal source of drinking water, and if contaminated, would create a significant hazard to public health. This action was taken under the authority of section 1424(e) of the Safe Drinking Water Act in response to a petition submitted to EPA by the Seattle-King County Department of Public Health on April 2, 1992. As a result of this determination, all federal financially assisted projects proposed in the designated area will be subject to EPA review to ensure that they do not create a significant hazard to public health.

EFFECTIVE DATE: This determination shall be promulgated for purposes of judicial review at 1:00 p.m. Eastern time on July 19, 1994.

ADDRESSES: The information upon which this determination is based is available to the public and may be inspected during normal business hours at the EPA Library, 10th Floor, Park Place Building, 1200 Sixth Avenue, Seattle, Washington 98101.

FOR FURTHER INFORMATION CONTACT: Scott E. Downey, Environmental Protection Specialist, Ground Water Section, WD–133, U.S. Environmental Protection Agency, Region 10, 1200 Sixth Avenue, Seattle, Washington 98101, 206–553–0682.

SUPPLEMENTARY INFORMATION: This action is being taken under the authority of section 1424(e) of the Safe Drinking Water Act (42 United States Code, 300f, 300h-3(e), Public Law 93–523). The information upon which EPA is issuing this final determination has been summarized in the "Support Document for Sole Source Aquifer Designation of the Vashon-Maury Island Aquifer System", EPA 910/K–94–002.

I. Background

Section 1424(e) of the Safe Drinking Water Act states:

> If the Administrator determines, on his own initiative or upon petition, that an area has an aquifer which is the sole or principal drinking water source for the area and which, if contaminated, would create a significant hazard to public health, he shall publish notice of that determination in the **Federal Register**. After the publication of any such notice, no commitment for federal financial assistance (through a grant, contract, loan guarantee, or otherwise) may be entered into for any project which the Administrator determines may contaminate such aquifer through a recharge zone so as to create a significant hazard to public health, but a commitment for federal assistance may, if authorized under another provision of law, be entered into to plan or design the project to assure that it will not so contaminate the aquifer.

Although EPA has the authority to initiate "sole source aquifer" designations, the Agency has a policy of acting only in response to petitions. Petitions may be submitted to EPA by any individual or organization and must address procedures and criteria outlined in EPA's "Sole Source Aquifer Designation Petitioner Guidance", EPA 440/6–87–003.

On April 2, 1992, EPA Region 10 received a petition from the Seattle-King County Department of Public Health requesting that EPA designate Vashon-Maury Island as a sole source aquifer. The petition was developed in cooperation with the Vashon-Maury Island Ground Water Advisory Committee and the Vashon-Maury Island Water Utilities Coordinating Committee. Recognizing the value of the aquifer as a present and future source of drinking water, the petition was submitted as an additional way to protect the Island's ground water resources.

EPA's initial review of the petition led to a request for additional hydrogeologic and water usage information. This information was subsequently submitted to EPA by the petitioner. On October 21, 1992, the petition was considered complete enough to undergo a more detailed technical review. The technical review was completed in April of 1994 and EPA's findings and basis for the proposed designation were documented in EPA's Support Document.

II. Basis for Determination

The Region 10 Administrator has determined that the Vashon-Maury Island Aquifer System meets all applicable sole source aquifer designation criteria established through Federal statute and EPA guidance documents, as follows:

(1) Sole or Principal Source of Drinking Water: Sole source aquifers must supply at least 50 percent of the drinking water to persons living in the area overlying the aquifer and in areas supplied by the aquifer. The Vashon-Maury Island Aquifer System supplies approximately 71 percent of the drinking water to persons living on the Island.

(2) Potential Public Health Hazard: Contamination of the sole source aquifer must create a significant hazard to public health. As the principal drinking water source for the area, contamination of the Vashon-Maury Island Aquifer System would create a significant hazard to public health.

(3) Definable Aquifer Boundaries: EPA guidance allows designations to be made for entire aquifers, hydrogeologically connected aquifers (aquifer systems), or part of an aquifer if that portion is hydrogeologically separated from the rest of the aquifer. The Vashon-Maury Aquifer System boundary is based on hydrogeological principles and EPA's interpretation of available data. The Island's hydrogeology is representative of an aquifer system, as data indicate that water from shallow aquifers infiltrates to underlying deeper aquifers. The sole source aquifer boundary is coincident with the shoreline of the Island, and at depth includes all geologic units that can supply significant quantities of drinking water to wells. This boundary is assumed because stratigraphic data are not available to fully map the vertical extent of the aquifer materials.

(4) No Alternative Source of Drinking Water: There can be no physical, legal, or economically feasible alternative source(s) of drinking water of sufficient volume that could replace the sole source aquifer, should it become contaminated. EPA has determined that there are no reasonably available alternative source(s) of drinking water that could replace the aquifer.

III. Description of the Vashon-Maury Island Aquifer System

Note: Information in this section represents an unfootnoted summary from EPA's Support Document, EPA 910/K–94–002.

Vashon-Maury Island is located near the southern end of Puget Sound in the southwestern corner of King County, Washington. The Island covers an area of 36.7 square miles and its population has been estimated at approximately 7,800 persons. Recorded data indicates an average rainfall of 46.53 inches.

The aquifer system is composed of both interbedded glacial and non-glacial deposits. In general, the water table elevation reflects the surface topography and the ground water moves radially outward from the interior to the shorelines of the Island.

The uppermost and most recent deposits (Quaternary Vashon unit) are mainly stratified sand and gravel overlying glacial till and sandy gravel

interbedded with medium and fine-grained sand. The Vashon unit contains a surficial aquifer comprised primarily of glacial till which has poor water-bearing characteristics, and the uppermost fresh water aquifer (Principal Aquifer) comprised of outwash sand and gravel beds. The Principal Aquifer is found at an elevation of between 0 and 400 feet above mean sea level. Recharge of the Principal Aquifer is probably highest along a north-south corridor of west-central Vashon Island, and is estimated to be approximately 9 million gallons per day. The Principal Aquifer supplies ninety-five percent of the wells located on the Island.

Underlying the Vashon unit are non-glacial deposits (Quaternary Olympia beds) generally consisting of thin-bedded sand and silt with local layers of gravel, massive silt and clayey silt. The Olympia beds serve as a leaky aquitard between the upper Principal Aquifer and the lower Deep Aquifer. The Deep Aquifer underlies the Olympia beds and consists of a variety of interbedded glacial tills, sand and gravel units and laminated silts and clays. The Deep Aquifer is located at an elevation of between about 100 to 300 feet below mean sea level. Recharge to the Deep Aquifer is estimated at between 1.73 and 3.46 million gallons per day.

Ground water quality data was sampled from 72 wells in the aquifer area. In general, deeper wells exhibited higher specific conductance values. Elevated chloride concentrations were found in near-shore wells on the northern and eastern edges of the Island. Ground water quality trend data is limited, but combined water system and spring data indicate that source water nitrate concentrations show a generally increasing trend.

The sand interbeds within the surficial glacial till deposits allow easy infiltration, and although discontinuous, make much of the Principal Aquifer vulnerable to contamination. The Deep Aquifer is also vulnerable to contamination from activities occurring on the land surface, as evidence suggests that it receives recharge from the Principal Aquifer.

Potential sources of contamination include landfill leachate, on-site sewage disposal systems, leaky sewer lines, underground storage tanks, agricultural chemicals, small hazardous waste generators, accidental spills, seawater intrusion, and improper household, forestry and farm practices.

The Island has one publicly-owned water well (the largest water system on the Island), at least six large private water systems, and more than 100 smaller water systems. Some purveyors use both surface water and ground water to supply their distribution system. In addition, private wells provide water to a considerable number of houses and businesses across the Island. It is estimated that 71% of the water supplied to households on the Island is from ground water and 29% is from surface water sources. There are no alternative sources of drinking water for the Island that can be physically, legally, and economically supplied.

IV. Project Reviews

Designation of a sole source aquifer authorizes EPA to review federal financially-assisted projects proposed within the designated area. The principal mechanism used by EPA Region 10 to identify projects for review are Memorandums of Understanding (MOUs) with federal funding agencies. These MOUs outline procedures for screening and referring projects to EPA in order to ensure that only projects which may have a significant impact to ground water quality are reviewed.

Most projects referred to EPA for review meet all federal, state, and local ground water protection standards and are approved without any additional conditions being imposed. Occasionally, site or project-specific concerns for ground water quality protection lead to specific recommendations or additional pollution prevention requirements as a condition of funding. In rare cases, federal funding has been denied when the applicant has been either unwilling or unable to modify the project.

Whenever feasible, EPA coordinates the review of proposed projects with other offices within EPA and with various federal, state, or local agencies that have a responsibility for ground water quality protection. Relevant information from these sources is given full consideration in the sole source aquifer review process. Such coordination can complement, support, and strengthen existing ground water protection mechanisms.

V. Public Comments

EPA issued a news release (April 12, 1994) and a public notice (April 14, 1994) to request comments and announce the proposed designation. Both stated that a public hearing would be held if sufficient interest were expressed to EPA in advance. No requests for a formal hearing were received and it was subsequently cancelled.

Five written comments were received prior to the expiration of the public comment period on June 1, 1994. Three letters were from Vashon Island residents and expressed support for the proposed designation. One letter was from the King County Department of Public Works, Roads and Engineering Division, and requested information and coordination of future federal financially-assisted road projects on the Island. Another letter was from the Bureau of Reclamation and stated there were no ongoing or proposed federal financially-assisted projects within the area. No controversial issues were raised as a result of this proposed action.

VI. Summary

This determination affects only the Vashon-Maury Island Aquifer System located in King County, Washington. As a result of this determination, all federal financially-assisted projects proposed in the designated area will be subject to EPA review to ensure that they do not create a significant hazard to public health.

Dated: June 17, 1994.

Chuck Clarke,

Regional Administrator, U.S. Environmental Protection Agency, Region 10.

[FR Doc. 94–16220 Filed 7–1–94; 8:45 am]

BILLING CODE 6560–50–P

federal register

Tuesday
July 5, 1994

Part III

Department of Health and Human Services

National Institutes of Health

Recombinant DNA Research: Actions Under the Guidelines; Notice

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Recombinant DNA Research: Actions Under the Guidelines

AGENCY: National Institutes of Health, PHS, DHHS.

ACTION: Notice of actions under the National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines).

SUMMARY: This notice sets forth four actions to be taken by the Director, National Institutes of Health (NIH), under the May 7, 1986, NIH Guidelines (51 FR 16958).

FOR FURTHER INFORMATION CONTACT: Additional information can be obtained from Dr. Nelson A. Wivel, Director, Office of Recombinant DNA Activities (ORDA), Office of Science Policy and Technology Transfer, National Institutes of Health, Building 31, room 4B11, Bethesda, Maryland 20892, (301) 496–9838.

SUPPLEMENTARY INFORMATION: Today four actions are being promulgated under the NIH Guidelines. These four proposed actions were published for comment in the **Federal Register** announcements of August 11, 1987 (52 FR 29800); April 18, 1988 (53 FR 12752); December 30, 1988 (53 FR 53262); April 29, 1991 (56 FR 19776); November 9, 1993 (58 FR 59612); and February 11, 1994 (59 FR 6702). These proposed actions were reviewed and recommended for approval by the NIH Recombinant DNA Advisory Committee (RAC) at its meetings on September 21, 1987; December 3, 1988; January 30, 1989; May 30–31, 1991; December 2–3, 1993; and March 3–4, 1994.

In accordance with Section IV-C-1-b of the NIH Guidelines, these actions have been found to comply with the NIH Guidelines and to present no significant risk to health or the environment.

A revised version of the NIH Guidelines is published in a separate section of the **Federal Register** following this announcement. These revised NIH Guidelines differ from the previous version promulgated on May 7, 1986 (51 FR 16958) by incorporating within them the major actions to the NIH Guidelines that were promulgated on August 24, 1987 (52 FR 31848); July 29, 1988 (53 FR 28819); October 26, 1988 (53 FR 43410); March 13, 1989 (54 FR 10508); March 1, 1990 (55 FR 7438); September 12, 1990 (55 FR 37565); July 18, 1991 (56 FR 33174); October 15, 1991 (56 FR 51784); November 21, 1991 (56 FR 58800); January 28, 1992 (57 FR 3212); April 22, 1992 (57 FR 14774); August 26, 1992 (57 FR 38734); February 18, 1993 (58 FR 9102); April 23, 1993 (58 FR 21738); September 13, 1993 (58 FR 47906); October 18, 1993 (58 FR 53814); and the changes that are promulgated in this announcement.

I. Background Information and Decision on Action Under the NIH Guidelines

A. Amendment to Sections II, III–C, III–D, V, Appendices C–I, and G and Addition of Appendix P, Physical and Biological Containment for Recombinant DNA Research Involving Plants, and Appendix Q, Physical and Biological Containment for Recombinant DNA Research Involving Animals of the NIH Guidelines

The NIH Guidelines were originally developed to cover research in laboratories in which recombinant DNA techniques were used. It is recognized today that these techniques are being used by scientists working with plants and large animals, and that procedures for containment of these plants and animals have not been specifically described in the NIH Guidelines. Institutional Biosafety Committees (IBCs) have requested guidance on the containment procedures that should be recommended for specific experiments with these organisms since they have the responsibility of approving such experiments under containment appropriate for the organisms. The principles of biological safety that are used to categorize experiments involving microorganisms, for example, are equally applicable to plants and animals. These safety procedures have been employed successfully for many years and have been recognized for their efficacy in biological containment.

Appendices P and Q are the result of several years of meetings and discussions involving research scientists and representatives from university, government, and industrial research sectors with expertise in several disciplines, including plant genetics, plant physiology, plant pathology, entomology, animal (including arthropod and aquatic species) physiology and reproduction, molecular biology, veterinary medicine, and human biomedical research. The Federal agencies involved in the development of Appendices P and Q include the NIH, the National Science Foundation (NSF), and the U.S. Department of Agriculture (USDA).

The process of developing Appendices P and Q was initiated when the USDA published an Advanced Notice of Proposed USDA Guidelines (USDA Guidelines) in the **Federal Register** on June 26, 1986 (51 FR 23367). This notice was followed by an announcement by the USDA regarding its intent to propose new guidelines for conducting all phases of research with domestic agriculture species, including both plants and animals modified through the application of genetic engineering techniques, in the **Federal Register** on December 9, 1986 (51 FR 44397). At that time, the NIH Guidelines did not include specific descriptions for containment conditions for research involving recombinant DNA containing whole plants and animals. The USDA convened a working group composed of university, government, and industrial scientists on December 13–14, 1986, with the purpose of discussing and redrafting guidelines for physical and biological containment of transgenic plant and animal species, and associated microorganisms. This meeting came to be known as the "Arlington House Workshop."

Participants of the "Arlington House Workshop," including former members of the RAC, agreed that the USDA Guidelines should be incorporated into the NIH Guidelines. The workshop participants noted that merging these two documents would offer the distinct advantage of providing a single comprehensive source of information regarding conduct of research involving organisms containing recombinant DNA and plants and animals exposed to microorganisms containing recombinant DNA.

A staff working group representing the Office of Recombinant DNA Activities, NIH, and the Cooperative State Research Service, USDA, held meetings during the following six months. This working group met with the purpose of revising the containment section and developing a final incorporated document for RAC review, approval by the NIH Director, and incorporation into the NIH Guidelines.

On June 28, 1987, and July 16, 1987, the RAC appointed the Working Group on Revision of the NIH Guidelines to meet and consider the draft documents, Appendices P and Q, and minor modifications to the NIH Guidelines, that would accommodate the proposed appendices. Appendices P and Q and the proposed revisions to the NIH Guidelines were published for public comment in the **Federal Register** on August 11, 1987 (52 FR 29800). Additional revisions to Appendices P and Q were proposed by the RAC and the Agricultural Research Service, USDA, at the September 17, 1987, RAC meeting. These modifications were

published for public comment in the Federal Register on December 30, 1988 (53 FR 53262). The RAC Working Group on Transgenic Animals proposed additional modifications to Appendices P and Q which were published for public comment in the Federal Register on April 18, 1988 (53 FR 12752). Further revisions were approved by the RAC at its January 30, 1989, meeting.

Throughout all of the meetings, discussions, and revisions, the intent of the Federal agencies and interested parties has been to describe working conditions that would minimize the risk to both the researcher and the environment from any possible harm or adverse effects due to the conduct of research involving recombinant DNA containing organisms.

On June 24, 1994, an Environmental Assessment of Appendices P and Q was completed by the NIH and USDA, and there was a finding of no significant impact. Copies of the Environmental Assessment are available from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, room 4B11, Bethesda, Maryland 20892, (301) 496–9838.

The actions are detailed in Section II—Summary of Actions. I accept these recommendations, and the NIH Guidelines will be amended accordingly.

B. Amendment to Sections I-C-1-b-(2) and Deletion of Section III–A–2 of the NIH Guidelines Regarding Deliberate Release

On December 6, 1990, the RAC Planning Subcommittee recommended that the requirement for RAC review of experiments involving deliberate environmental release of organisms containing recombinant DNA be eliminated from the NIH Guidelines. This recommendation reflects the fact that the Federal regulatory agencies, the USDA, and the Environmental Protection Agency (EPA), are responsible for the review and approval of environmental release experiments. The proposed amendment was published for public comment in the Federal Register on April 29, 1991 (56 FR 19776). The RAC reviewed and recommended approval of the proposed amendment at its May 30–31, 1991, meeting.

The actions are detailed in Section II—Summary of Actions. I accept these recommendations, and the NIH Guidelines will be amended accordingly.

C. Amendments to Sections I, III, IV, and V, and Appendix M of the NIH Guidelines Regarding NIH/ORDA Review and Approval of Certain Categories of Human Gene Transfer Experiments That Qualify for the Accelerated Review Process

On December 3, 1993, and March 3–4, 1994, the Working Group on Accelerated Review Protocols presented an overview of the proposed amendments to the NIH Guidelines. The proposed amendments will: (1) Establish an accelerated review process for certain categories of human gene transfer experiments, (2) allow the NIH/Office of Recombinant DNA Activities to assign the appropriate review category to all human gene transfer proposals that are submitted in compliance with the NIH Guidelines, (3) allow the NIH/Office of Recombinant DNA Activities to approve those categories of human gene transfer experiments that qualify for the accelerated review process in consultation with the Chair and one or more RAC members, as necessary, and (4) exempt certain experiments involving the transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects which are not covered by Section V–U. All human gene transfer experiments approved by the NIH/Office of Recombinant DNA Activities through the accelerated review process will be provided in a report by the RAC Chair at the next regularly scheduled RAC meeting and will be included in the list of approved experiments which is available from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838.

The proposed amendments were published for public comment in the Federal Register on November 9, 1993 (58 FR 59612) and February 11, 1994 (59 FR 6702). The RAC reviewed and unanimously recommended approval of the proposed amendments at its March 3–4, 1994, meeting.

The actions are detailed in Section II—Summary of Actions. I accept these recommendations, and the NIH Guidelines will be amended accordingly.

D. Amendments to Section V–U of the NIH Guidelines Regarding Recombinant DNA Vaccines

On March 3, 1994, the Working Group on Vaccines presented an overview of the proposed amendment to the footnote in Section V–U. The proposed amendment will define those categories of experiments involving the administration of recombinant DNA vaccines that are exempt from RAC review and NIH and Institutional Biosafety Committee approval.

The proposed amendment was published for public comments in the Federal Register on February 11, 1994 (59 FR 6702). The proposed amendment was revised by the RAC at its March 3–4, 1994, meeting. The revised amendment was unanimously approved.

The action is detailed in Section II—Summary of Actions. I accept this recommendation, and the NIH Guidelines will be amended accordingly.

II. Summary of Actions

A. Amendment to Section I, Scope of the NIH Guidelines

The amended version of Section I reads as follows:

Section I. Scope of the NIH Guidelines

Section I–A. Purpose

The purpose of the NIH Guidelines is to specify practices for constructing and handling: (i) Recombinant deoxyribonucleic acid (DNA) molecules, and (ii) organisms and viruses containing recombinant DNA molecules.

Section I–A–1. Any recombinant DNA experiment, which according to the NIH Guidelines requires approval by the NIH, must be submitted to the NIH or to another Federal agency that has jurisdiction for review and approval. Once approval, or other applicable clearances, has been obtained from a Federal agency other than the NIH (whether the experiment is referred to that agency by the NIH or sent directly there by the submitter), the experiment may proceed without the necessity for NIH review or approval (see exceptions in Sections I–A–2 and I–A–3).

Section I–A–2. Certain experiments that involve the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects (see Section V–U) shall be considered Major Actions (see Section IV-C-1-b-(1)), and shall require RAC review and NIH Director approval, if determined by NIH/ORDA in consultation with the RAC Chair and/or one or more RAC members, as necessary, to: (i) Represent novel characteristics (e.g., target disease or vector), (ii) represent an uncertain degree of risk to human health or the environment, or (iii) contain information determined to require further public review (see Section III–A–2).

Section I–A–3. Experiments involving the transfer of recombinant DNA to one or more human subjects that are not considered under Section III–A–2 may qualify for Accelerated Review (see Section III–B–2 and Appendix M–V) and will be considered as Minor Actions (see Section IV-C-1-b-(2)-(a)). Actions that qualify for Accelerated Review will be reviewed and approved by NIH/ORDA in consultation with the RAC Chair and/or one or more RAC members, as necessary.

Certain experiments involving the transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects (see Section V–U) may be considered exempt from RAC and/or NIH/ORDA review and/or NIH Director approval and only require registration with NIH/ORDA (see Section III–C–7).

Section I–B. Definition of Recombinant DNA Molecules

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

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

Genomic DNA of plants and bacteria that have acquired a transposable element, even if the latter was donated from a recombinant vector no longer present, are not subject to the NIH Guidelines unless the transposon itself contains recombinant DNA.

Section I–C. General Applicability

Section I–C–1. The NIH Guidelines are applicable to:

Section I-C-1-a. All recombinant DNA research within the United States (U.S.) or its territories that is conducted at or sponsored by an institution that receives any support for recombinant DNA research from the NIH, including research performed directly by the NIH. An individual who receives support for research involving recombinant DNA must be associated with or sponsored by an institution that assumes the responsibilities assigned in the NIH Guidelines.

Section I-C-1-b. All recombinant DNA research performed abroad: Specifically:

Section I-C-1-b-(1). Research supported by NIH funds.

Section I-C-1-b-(2). If they involve testing in humans of materials containing recombinant DNA developed with NIH funds and if the institution that developed those materials sponsors or participates in those projects. Participation includes research collaboration or contractual agreements, not mere provision of research materials.

Section I-C-1-b-(3). If the host country has established rules for the conduct of recombinant DNA research, then the research must be in compliance with those rules. If the host country does not have such rules, the proposed research must be reviewed and approved by an NIH-approved Institutional Biosafety Committee or equivalent review body and accepted in writing by an appropriate national governmental authority of the host country. The safety practices that are employed abroad must be reasonably consistent with the NIH Guidelines.

Section I-D. General Definitions

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

Section I-D–1. An 'institution' is any public or private entity (including Federal, state, and local government agencies).

Section I-D–2. An 'Institutional Biosafety Committee' is a committee that: (i) meets the requirements for membership specified in Section IV-B–2, and (ii) reviews, approves, and oversees projects in accordance with the responsibilities defined in Section IV-B–2.

Section I-D–3. The 'Office of Recombinant DNA Activities (ORDA)' is the office within the NIH that is responsible for: (i) Reviewing and coordinating all activities relating to the NIH Guidelines, and (ii) performing other duties as defined in Section IV-C–3.

Section I-D–4. The 'Recombinant DNA Advisory Committee' is the public advisory committee that advises the Department of Health and Human Services (DHHS) Secretary, the DHHS Assistant Secretary for Health, and the NIH Director concerning recombinant DNA research. The RAC shall be constituted as specified in Section IV-C–2.

Section I-D–5. The 'NIH Director' is the Director of the National Institutes of Health, or any other officer or employee of NIH to whom authority has been delegated.

Section I-D–6. 'Deliberate release' is defined as a planned introduction of recombinant DNA-containing microorganisms, plants, or animals into the environment.

B. Amendment to Section II, Containment, of the NIH Guidelines

The amended version of Section II reads as follows:

Section II. Containment

Effective biological safety programs have been operative in a variety of laboratories for many years. Considerable information already exists about the design of physical containment facilities and selection of laboratory procedures applicable to organisms carrying recombinant DNA (see Section V-A). The existing programs rely upon mechanisms that 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 that are applied in varying degrees according to the estimated biohazard. Four biosafety levels are described in Appendix G. These biosafety levels consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities appropriate for the operations performed and are based on the potential hazards imposed by the agents used and for the laboratory function and activity. Biosafety Level 4 provides the most stringent containment conditions, Biosafety Level 1 the least stringent.

Experiments involving recombinant DNA lend themselves to a third containment mechanism, namely, the application of highly specific biological barriers. Natural barriers exist that 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. Vectors, which provide the means for recombinant DNA and/or host cell replication, can be genetically designed to decrease, by many orders of magnitude, the probability of dissemination of recombinant DNA outside the laboratory (see Appendix I).

Since these three means of containment are complementary, different levels of containment can be established that apply various combinations of the physical and biological barriers along with a constant use of standard practices. Categories of containment are considered separately in order that such combinations can be conveniently expressed in the NIH Guidelines.

Physical containment conditions within laboratories, described in Appendix G, may not always be appropriate for all organisms because of their physical size, the number of organisms needed for an experiment, or the particular growth requirements of the organism. Likewise, biological containment for microorganisms described in Appendix I may not be appropriate for all organisms, particularly higher eukaryotic organisms. However, significant information exists about the design of research facilities and experimental procedures that are applicable to organisms containing recombinant DNA that is either integrated into the genome or into microorganisms associated with the higher organism as a symbiont, pathogen, or other relationship. This information describes facilities for physical containment of organisms used in non-traditional laboratory settings and special practices for limiting or excluding the unwanted establishment, transfer of genetic information, and dissemination of organisms beyond the intended location, based on both physical and biological containment principles. Research conducted in accordance with these conditions effectively confines the organism.

For research involving plants, four biosafety levels (BL1–P through BL4–P) are described in Appendix P. BL1–P is designed to provide a moderate level of containment for experiments for which there is convincing biological evidence that precludes the possibility of survival, transfer, or dissemination of recombinant DNA into the environment, or in which there is no recognizable and predictable risk to the environment in the event of accidental release. BL2–P is designed to provide a greater level of containment for experiments involving plants and certain associated organisms in which there is a recognized possibility of survival, transmission, or dissemination of recombinant DNA containing organisms, but the consequence of such an inadvertent release has a predictably minimal biological impact. BL3–P and BL4–P describe additional containment conditions for research with plants and certain pathogens and other organisms that require special containment because of their recognized potential for significant detrimental impact on managed or natural ecosystems. BL1–P relies upon accepted scientific practices for conducting research in most ordinary greenhouse or growth chamber facilities and incorporates accepted procedures for good pest control and cultural practices. BL1–P facilities and procedures provide a modified and protected environment for the propagation of plants and microorganisms associated with the plants and a degree of containment that adequately controls the potential for release of biologically viable plants, plant parts, and microorganisms associated with them. BL2–P and BL3–P rely upon accepted scientific practices for conducting research in greenhouses with organisms infecting or infesting plants in a manner that minimizes or prevents inadvertent contamination of plants within or surrounding the greenhouse. BL4–P describes facilities and practices known to provide containment of certain exotic plant pathogens.

For research involving animals, which are of a size or have growth requirements that preclude the use of conventional primary containment systems used for small laboratory animals, four biosafety levels (BL1–N through BL4–N) are described in Appendix Q. BL1–N describes containment for animals that have been modified by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant DNA-modified microorganisms and is designed to eliminate the possibility of sexual transmission of the modified genome or transmission of recombinant DNA-derived viruses known to be transmitted from animal parent to offspring only by sexual reproduction. Procedures, practices, and facilities follow classical methods of avoiding genetic exchange between animals. BL2–N describes containment which is used for transgenic animals associated with recombinant DNA-derived organisms and is designed to eliminate the possibility of vertical or horizontal transmission. Procedures, practices, and facilities follow classical methods of avoiding genetic exchange between animals or controlling arthropod transmission. BL3–N and BL4–N describe higher levels of containment for research with certain transgenic animals involving agents which pose recognized hazard.

In constructing the NIH 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. These definitions do not take into account all existing and anticipated information on special procedures that will allow particular experiments to be conducted under different conditions than indicated here without affecting risk. Individual investigators and Institutional Biosafety Committees are urged to devise simple and more effective containment procedures and to submit recommended changes in the NIH Guidelines to permit the use of these procedures."

C. Amendment to Section III, Experiments Covered by the NIH Guidelines

The previous version of Section III-A–2 will be deleted as follows:

Section III-A–2. Deliberate release into the environment of any organism containing recombinant DNA except those listed below. The term 'deliberate release' is defined as a planned introduction of recombinant DNA-containing microorganisms, plants, or animals into the environment.

Section III-A–2-a. Introduction conducted under conditions considered to be accepted scientific practices in which there is adequate evidence of biological and/or physical control of the recombinant DNA-containing organisms. The nature of such evidence is described in Appendix L.

Section III-A–2-b. Deletion derivatives and single base changes not otherwise covered by the NIH Guidelines.

Section III-A–2-c. For extrachromosomal elements and microorganisms (including viruses), rearrangements and amplifications within a single genome. Rearrangements involving the introduction of DNA from different strains of the same species would not be covered by this exemption."

The amended version of Section III reads as follows:

Section III. Experiments Covered by the NIH Guidelines.

This section describes five categories of experiments involving recombinant DNA: (i) Those that require RAC review and NIH and Institutional Biosafety Committee approval before initiation (see Section III-A), (ii) those that require NIH/ORDA and Institutional Biosafety Committee approval before initiation (see Section III-B); (iii) those that require Institutional Biosafety Committee approval before initiation (see Section III-C), (iv) those that require Institutional Biosafety Committee notification simultaneous with initiation (see Section III-D), and (v) those that are exempt from the NIH Guidelines (see Section III-E).

Note: If an experiment falls into either Section III-A or Section III-B and one of the other categories, the rules pertaining to Section III-A or Section III-B shall be followed. If an experiment falls into Section III-E and into either Sections III-C or III-D categories as well, the experiment is considered exempt from the NIH Guidelines.

Any change in containment level, which is different from those specified in the NIH Guidelines, may not be initiated without the express approval of NIH/ORDA (see Minor Actions, Section IV-C-1-b-(2) and its subsections).

Section III–A. Experiments That Require Institutional Biosafety Committee Approval, RAC Review, and NIH Approval Before Initiation

Experiments in this category are considered Major Actions (see Section IV-C-1-b-(1)) and cannot be initiated without submission of relevant information on the proposed experiment to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838, the publication of the proposal in the Federal Register for 15 days of comment, reviewed by the RAC, and specific approval by the NIH (not applicable for Expedited Review single patient human gene transfer experiments considered under Appendix M-VI). The containment conditions for such experiments will be recommended by the RAC and set by the NIH at the time of approval. Such experiments require Institutional Biosafety Committee approval before initiation. Specific experiments already approved are included in Appendix D which may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838.

Section III–A–1. Deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally (see Section V–B), if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture.

Section III–A–2. Certain experiments involving the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects (see Section V–U) shall be considered Major Actions (see Section IV–C–1–b–(1) and Appendix M–III), and shall require RAC review and NIH Director approval, if determined by NIH/ORDA, in consultation with the RAC Chair and one or more RAC members, as necessary, to: (i) represent novel characteristics (e.g., target disease or vector), (ii) represent an uncertain degree of risk to human health or the environment, or (iii) contain information determined to require further public review. The requirement for RAC review shall not be considered to preempt any other required review or approval of experiments with one or more human subjects. Relevant Institutional Biosafety Committee and Institutional Review Board reviews and approvals of the proposal should be completed before submission to NIH. Certain experiments involving deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects may qualify for the Accelerated Review process (see Section III–B–2). Certain categories of experiments involving the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects and that are not covered by Section V–U, may be considered exempt from RAC and/or NIH/ORDA review and/or NIH Director approval and only require registration with NIH/ORDA (see Section III–C–7).

Section III–B. Experiments That Require NIH/ORDA and Institutional Biosafety Committee Approval Before Initiation

Section III–B–1. Experiments Involving the Cloning of Toxin Molecules with LD_{50} of Less Than 100 Nanograms per Kilogram Body Weight

Deliberate formation of recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, and *Shigella dysenteriae* neurotoxin). Specific approval has been given for the cloning in *Escherichia coli* K–12 of DNA containing genes coding for the biosynthesis of toxic molecules which are lethal to vertebrates at 100 nanograms to 100 micrograms per kilogram body weight. Specific experiments already approved under this section may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838.

Section III–B–1–(a). Experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH/ORDA. The containment conditions for such experiments will be determined by NIH/ORDA in consultation with ad hoc experts. Such experiments require Institutional Biosafety Committee approval before initiation (see Section IV–B–2–b–(1)).

Section III–B–2. Accelerated Review of Human Gene Transfer Experiments

As determined by NIH/ORDA, in consultation with the RAC Chair and one or more RAC members, as necessary, certain categories of human gene transfer experiments may be considered as Minor Actions and qualify for Accelerated Review and approval (see Section IV–C–1–b–(2)–(a), Appendix M–III–A, and Appendix M–V). The RAC Chair will present a report of all NIH/ORDA approved human gene transfer protocols at the next regularly scheduled RAC meeting. If NIH/ORDA determines that an experiment does not qualify for the Accelerated Review process, the Principal Investigator must submit the proposal for full RAC review ≥ 8 weeks prior to the next scheduled RAC meeting (See Section III–A–2).

Section III–B–3. Minor Modifications to Human Gene Transfer Experiments

A minor modification in a human gene transfer protocol is a modification that does not significantly alter the basic design of the protocol and that does not increase risk to human subjects or the environment. After approval has been obtained by the relevant Institutional Biosafety Committee and Institutional Review Board, NIH/ORDA will consider the change in consultation with the RAC Chair and one or more RAC members, as necessary. Submit minor modifications to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838. The RAC Chair will provide a report on any such approvals at the next regularly scheduled RAC meeting.

Section III–C. Experiments That Require Institutional Biosafety Committee Approval Before Initiation

Prior to the initiation of an experiment that falls into this category, the Principal Investigator must submit a registration document to the Institutional Biosafety Committee which contains the following information: (i) The source(s) of DNA; (ii) the nature of the inserted DNA sequences; (iii) the host(s) and vector(s) to be used; (iv) if an attempt will be made to obtain expression of a foreign gene, and if so, indicate the protein that will be produced; and (v) the containment conditions that will be implemented as specified in the NIH Guidelines. For experiments in this category, the registration document shall be dated, signed by the Principal Investigator, and filed with the Institutional Biosafety Committee. The Institutional Biosafety Committee shall review and approve all

experiments in this category prior to their initiation. Requests to decrease the level of containment specified for experiments in this category will be considered by NIH (see Section IV–C–1–b–(2)–(c)).

Section III–C–1. Experiments Using Human or Animal Pathogens (Class 2, Class 3, Class 4, or Class 5 Agents (See Section V–A) as Host-Vector Systems

Section III–C–1–a. Experiments involving the introduction of recombinant DNA into Class 2 agents shall be conducted at Biosafety Level (BL) 2 containment. Experiments with such agents shall be conducted with whole animals at BL2 or BL2–N (Animals) containment.

Section III–C–1–b. Experiments involving the introduction of recombinant DNA into Class 3 agents shall be conducted at BL3 containment. Experiments with such agents shall be conducted with whole animals at BL3 or BL3–N containment.

Section III–C–1–c. Experiments involving the introduction of recombinant DNA into Class 4 agents shall be conducted at BL4 containment. Experiments with such agents shall be conducted with whole animals at BL4 or BL4–N containment.

Section III–C–1–d. Containment conditions for experiments involving the introduction of recombinant DNA into Class 5 agents shall be set on a case-by-case basis following NIH/ORDA review. A U.S. Department of Agriculture permit is required for work with Class 5 agents (see Sections V–R and V–T). Experiments with such agents shall be conducted with whole animals at BL4 or BL4–N containment.

Section III–C–2. Experiments in Which DNA From Human or Animal Pathogens (Class 2, Class 3, Class 4, or Class 5 Agents (See Section V–A) is Cloned Into Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems

Section III–C–2–a. Experiments in which DNA from Class 2 or Class 3 agents (see Section V–A) is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment. Experiments in which DNA from Class 4 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment after demonstration that only a totally and irreversibly defective fraction of the agent's genome is present in a given recombinant. In the absence of such a demonstration, BL4 containment shall be used. The Institutional Biosafety Committee may approve the specific lowering of containment for particular experiments to BL1. Many experiments in this category are exempt from the NIH Guidelines (see Section III–E). Experiments involving the formation of recombinant DNA for certain genes coding for molecules toxic for vertebrates require NIH/ORDA approval (see Section III–B–1) or shall be conducted under NIH specified conditions as described in Appendix F.

Section III–C–2–b. Containment conditions for experiments in which DNA from Class 5 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes shall be determined by NIH/ORDA following a case-by-case review. A U.S. Department of Agriculture permit is required for work with Class 5 agents (see Sections V–R and V–T).

Section III–C–3. Experiments Involving the Use of Infectious Animal or Plant DNA or RNA Viruses or Defective Animal or Plant DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems

Caution: Special care should be used in the evaluation of containment levels for experiments which are likely to either enhance the pathogenicity (e.g., insertion of a host oncogene) or to extend the host range (e.g., introduction of novel control elements) of viral vectors under conditions that permit a productive infection. In such cases, serious consideration should be given to increasing physical containment by at least one level.

Note: Recombinant DNA or RNA molecules derived therefrom, which contain less than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family (see Section V–Q) being considered identical (see Section V–S), are considered defective and may be used in the absence of helper under the conditions specified in Section III–D–1.

Section III–C–3–a. Experiments involving the use of infectious or defective Class 2 animal viruses (see Section V–A, Appendix B–II, and Appendix B–II–E) in the presence of helper virus may be conducted at BL2.

Section III–C–3–b. Experiments involving the use of infectious or defective Class 3 animal viruses (see Section V–A and Appendix B–III–D) in the presence of helper virus may be conducted at BL3.

Section III–C–3–c. Experiments involving the use of infectious or defective Class 4 animal viruses (see Section V–A and Appendix B–IV–D) in the presence of helper virus may be conducted at BL4.

Section III–C–3–d. Experiments involving the use of infectious or defective Class 5 viruses (see Section V–A and Appendix B–V) in the presence of helper virus shall be determined on a case-by-case basis following NIH/ORDA review. A U.S. Department of Agriculture permit is required for work with Class 5 agents (see Sections V–R and V–T).

Section III–C–3–e. Experiments involving the use of infectious or defective animal or plant viruses in the presence of helper virus are not covered in Sections III–C–3–a through III–C–3–d and may be conducted at BL1.

Section III–C–4. Experiments Involving Whole Animals

This section covers experiments involving whole animals in which the animal's genome has been altered by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant DNA-modified microorganisms tested on whole animals. For the latter, other than viruses which are only vertically transmitted, the experiments may not be conducted at BL1–N containment. A minimum containment of BL2 or BL2–N is required.

Caution—Special care should be used in the evaluation of containment conditions for some experiments with transgenic animals. For example, such experiments might lead to the creation of novel mechanisms or increased transmission of a recombinant pathogen or production of undesirable traits in the host animal. In such cases, serious consideration should be given to increasing the containment conditions.

Section III–C–4–a. Recombinant DNA, or DNA or RNA molecules derived therefrom, from any source except for greater than two-thirds of eukaryotic viral genome may be transferred to any non-human vertebrate or any invertebrate organism and propagated under conditions of physical containment comparable to BL1 or BL1–N and appropriate to the organism under study (see Section V–B).

Animals that contain sequences from viral vectors, which do not lead to transmissible infection either directly or indirectly as a result of complementation or recombination in animals, may be propagated under conditions of physical containment comparable to BL1 or BL1–N and appropriate to the organism under study. Experiments involving the introduction of other sequences from eukaryotic viral genomes into animals are covered under Section III–C–4–b. For experiments involving recombinant DNA-modified Class 2, 3, 4, or 5 organisms, see Section V–A. It is important that the investigator

demonstrate that the fraction of the viral genome being utilized does not lead to productive infection. A U.S. Department of Agriculture permit is required for work with Class 5 agents (see Section V–R and V–T).

Section III–C–4–b. For experiments involving recombinant DNA, or DNA or RNA derived therefrom, involving whole animals, including transgenic animals, and not covered by Sections III–C–1 or III–C–4–a, the appropriate containment shall be determined by the Institutional Biosafety Committee.

Section III–C–5. Experiments Involving Whole Plants

Experiments to genetically engineer plants by recombinant DNA methods, to use such plants for other experimental purposes (e.g., response to stress), to propagate such plants, or to use plants together with microorganisms or insects containing recombinant DNA, may be conducted under the containment conditions described in Sections III–C–5–a through III–C–5–e. If experiments involving whole plants are not described in Section III–C–5 and do not fall under Sections III–A, III–B, or III–E, they are included in Section III–D.

Note. For recombinant DNA experiments falling under Sections III–C–5–a through III–C–5–d, physical containment requirements may be reduced to the next lower level by appropriate biological containment practices, such as conducting experiments on a virus with an obligate insect vector in the absence of that vector or using a genetically attenuated strain.

Section III–C–5–a. BL3–P (Plants) or BL2–P + biological containment is recommended for experiments involving most exotic (see Section V–W) infectious agents with recognized potential for serious detrimental impact on managed or natural ecosystems when recombinant DNA techniques are associated with whole plants.

Section III–C–5–b. BL3–P or BL2–P + biological containment is recommended for experiments involving plants containing cloned genomes of readily transmissible exotic (see Section V–W) infectious agents with recognized potential for serious detrimental effects on managed or natural ecosystems in which there exists the possibility of reconstituting the complete and functional genome of the infectious agent by genomic complementation *in planta*.

Section III–C–5–c. BL4–P containment is recommended for experiments with a small number of readily transmissible exotic (see Section V–W) infectious agents, such as the soybean rust fungus (*Phakospora pachyrhizi*) and maize streak or other viruses in the presence of their specific arthropod vectors, that have the potential of being serious pathogens of major U.S. crops.

Section III–C–5–d. BL3–P containment is recommended for experiments involving sequences encoding potent vertebrate toxins introduced into plants or associated organisms. Recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD_{50} of <100 nanograms per kilogram body weight fall under Section III–B–1 and require NIH/ORDA and Institutional Biosafety Committee approval before initiation.

Section III–C–5–e. BL3–P or BL2–P + biological containment is recommended for experiments with microbial pathogens of insects or small animals associated with plants if the recombinant DNA-modified organism has a recognized potential for serious detrimental impact on managed or natural ecosystems.

Section III–C–6. Experiments Involving More than 10 Liters of Culture

The appropriate containment will be decided by the Institutional Biosafety Committee. Where appropriate, Appendix K, Physical Containment for Large Scale Uses of Organisms Containing Recombinant DNA Molecules, shall be used. Appendix K describes containment conditions Good Large Scale Practice through BL3–Large Scale.

Section III–C–7. Human Gene Transfer Experiments Not Covered by Sections III–A–2, III–B–2, III–B–3, and Not Considered Exempt Under Section V–U

Certain experiments involving the transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects that are not covered by Sections III–A–2, III–B–2, III–B–3, and that are not considered exempt under Section V–U must be registered with NIH/ORDA. The relevant Institutional Biosafety Committee and Institutional Review Board must review and approve all experiments in this category prior to their initiation.

Section III–D. Experiments That Require Institutional Biosafety Committee Notice Simultaneous With Initiation

Experiments not included in Sections III–A, III–B, III–C, III–E, and their subsections are considered in Section III–D. All such experiments may be conducted at BL1 containment. For experiments in this category, a registration document (see Section III–C) shall be dated and signed by the investigator and filed with the local Institutional Biosafety Committee at the time the experiment is initiated. The Institutional Biosafety Committee reviews and approves all such proposals, but Institutional Biosafety Committee review and approval prior to initiation of the experiment is not required (see Section IV–A). For example, experiments in which all components derived from non-pathogenic prokaryotes and non-pathogenic lower eukaryotes fall under Section III–D and may be conducted at BL1 containment.

Section III–D–1. Experiments Involving the 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 (see Section V–Q) being considered identical (see Section V–S)) may be propagated and maintained in cells in tissue culture using BL1 containment. For such experiments, it must be demonstrated that the cells lack helper virus for the specific Families of defective viruses being used. If helper virus is present, procedures specified under Section III–C–3 should be used. The DNA may contain fragments of the genome of viruses from more than one Family but each fragment shall be less than two-thirds of a genome.

Section III–D–2. Experiments Involving Whole Plants

This section covers experiments involving recombinant DNA-modified whole plants, and/or experiments involving recombinant DNA-modified organisms associated with whole plants, except those that fall under Section III–A, III–B, III–C, or III–E. It should be emphasized that knowledge of the organisms and judgment based on accepted scientific practices should be used in all cases in selecting the appropriate level of containment. For example, if the genetic modification has the objective of increasing pathogenicity or converting a non-pathogenic organism into a pathogen, then a higher level of containment may be appropriate depending on the organism, its mode of dissemination, and its target organisms. By contrast, a lower level of containment may be appropriate for small animals associated with many types of recombinant DNA-modified plants.

Section III–D–2–a. BL1–P is recommended for all experiments with recombinant DNA-containing plants and

plant-associated microorganisms not covered in Section III-D–2-b or other sections of the NIH Guidelines. Examples of such experiments are those involving recombinant DNA-modified plants that are not noxious weeds or that cannot interbreed with noxious weeds in the immediate geographic area, and experiments involving whole plants and recombinant DNA-modified non-exotic (see Section V-W) microorganisms that have no recognized potential for rapid and widespread dissemination or for serious detrimental impact on managed or natural ecosystems (e.g., *Rhizobium* spp. and *Agrobacterium* spp.).

Section III-D–2-b. BL2–P or BL1–P + biological containment is recommended for the following experiments:

Section III-D–2-b-(1). Plants modified by recombinant DNA that are noxious weeds or can interbreed with noxious weeds in the immediate geographic area.

Section III-D–2-b-(2). Plants in which the introduced DNA represents the complete genome of a non-exotic infectious agent (see Section V-W).

Section III-D–2-b-(3). Plants associated with recombinant DNA-modified non-exotic microorganisms that have a recognized potential for serious detrimental impact on managed or natural ecosystems (see Section V-W).

Section III-D–2-b-(4). Plants associated with recombinant DNA-modified exotic microorganisms that have no recognized potential for serious natural ecosystems (see Section V-W).

Section III-D–2-b-(5). Experiments with recombinant DNA-modified arthropods or small animals associated with plants, or with arthropods or small animals with recombinant DNA-modified microorganisms associated with them if the recombinant DNA-modified microorganisms have no recognized potential for serious detrimental impact on managed or natural ecosystems (see Section V-W).

Section III-E. Exempt Experiments

The following recombinant DNA molecules are exempt from the NIH Guidelines and registration with the Institutional Biosafety Committee is not required:

Section III-E–1. Those that are not in organisms or viruses.

Section III-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.

Section III-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.

Section III-E–4. Those that consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).

Section III-E–5. Those 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 NIH Director with advice of the RAC after appropriate notice and opportunity for public comment (see Section IV-C–1-b-(1)-(c)). See Appendices A-I through A-VI for a list of natural exchangers that are exempt from the NIH Guidelines.

Section III-E–6. Those that do not present a significant risk to health or the environment (see Section IV-C–1-b-(1)-(c)), as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment. See Appendix C for other classes of experiments which are exempt from the NIH Guidelines."

D. Amendment to Section IV, Roles and Responsibilities of the NIH Guidelines

The amended version of Section IV-C–1-b reads as follows:

Section IV-C–1-b. Specific Responsibilities [NIH Director]

In carrying out the responsibilities set forth in this section, the NIH Director, or a designee shall weigh each proposed action through appropriate analysis and consultation to determine whether it complies with the NIH Guidelines and presents no significant risk to health or the environment.

Section IV-C–1-b-(1). Major Actions

To execute Major Actions, the NIH Director shall seek the advice of the RAC and provide an opportunity for public and Federal agency comment. Specifically, the Notice of Meeting and Proposed Actions to the NIH Guidelines shall be published in the **Federal Register** at least 15 days before the RAC meeting (not applicable for Expedited Review single patient human gene transfer experiments considered under Appendix M-VI). The NIH Director's decision, at his/her discretion, may be published in the **Federal Register** for 15 days of comment before final action is taken. The NIH Director's final decision, along with responses to public comments, shall be published in the **Federal Register**. The RAC and Institutional Biosafety Committee Chairs shall be notified of the following decisions:

Section IV-C–1-b-(1)-(a). Changing containment levels for types of experiments that are specified in the NIH Guidelines when a Major Action is involved;

Section IV-C–1-b-(1)-(b). Assigning containment levels for types of experiments that are not explicitly considered in the NIH Guidelines when a Major Action is involved;

Section IV–C–1–b–(1)–(c). Promulgating and amending a list of classes of recombinant DNA molecules to be exempt from the NIH 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;

Section IV–C–1–b–(1)–(d). Permitting experiments specified by Section III–A;

Section IV–C–1–b–(1)–(e). Certifying new host-vector systems with the exception of minor modifications of already certified systems (the standards and procedures for certification are described in Appendix I–II). Minor modifications constitute (e.g., those of minimal or no consequence to the properties relevant to containment); and

Section IV–C–1–b–(1)–(f). Adopting other changes in the NIH Guidelines.

Section IV–C–1–b–(2). Minor Actions

NIH/ORDA shall carry out certain functions as delegated to it by the NIH Director (see Section IV–C–3). Minor Actions (as determined by NIH/ORDA in consultation with the RAC Chair and one or more RAC members, as necessary) will be transmitted to the RAC and Institutional Biosafety Committee Chairs:

Section IV–C–1–b–(2)–(a). Reviewing and approving certain experiments involving the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects that qualify for the Accelerated Review process (see Section III–B–2);

Section IV–C–1–b–(2)–(b). Reviewing and approving minor changes to human gene transfer protocols under Section III–A–2 and III–B–2;

Section IV–C–1–b–(2)–(c). Changing containment levels for experiments that are specified in Section III;

Section IV–C–1–b–(2)–(d). Assigning containment levels for experiments not explicitly considered in the NIH Guidelines; and

Section IV–C–1–b–(2)–(e). Revising the Classification of Etiologic Agents for the purpose of these NIH Guidelines (see Section V–A).

Section IV–C–1–b–(2)–(f). Interpreting the NIH Guidelines for experiments to which the NIH Guidelines do not specifically assign containment levels;

Section IV–C–1–b–(2)–(g). Setting containment under Sections III–C–1–d and III–C–2–b;

Section IV–C–1–b–(2)–(h). Approving minor modifications of already certified host-vector systems (the standards and procedures for such modifications are described in Appendix I–II);

Section IV–C–1–b–(2)–(i). Decertifying already certified host-vector systems;

Section IV–C–1–b–(2)–(j). Adding new entries to the list of molecules toxic for vertebrates (see Appendix F); and

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

The amended version of Section IV–C–2 reads as follows:

Section IV–C–2. Recombinant DNA Advisory Committee (RAC). The RAC shall be responsible for advising the Director, NIH, on the actions listed in Section IV–C–1–b–(1).

The amended version of Section IV–C–3 reads as follows:

Section IV–C–3. Office of Recombinant DNA Activities (ORDA)

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 Officers, 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 NIH Director, including those authorities described in Section IV–C–1–b–(2). ORDA's responsibilities include, but are not limited to the following:

Section IV–C–3–a. Reviewing and approving experiments in conjunction with ad hoc experts involving the cloning of genes encoding for toxin molecules that are lethal for vertebrates at an LD_{50} ≤100 nanograms per kilogram body weight in organisms other than *Escherichia coli* K–12 (see Section III–B–1 and Appendices F–I and F–II);

Section IV–C–3–b. Reviewing and approving certain experiments involving the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects, in consultation with the RAC Chair and one or more RAC members, as necessary, that qualify for the Accelerated Review process (see Section III–B–2);

Section IV–C–3–c. Reviewing and approving minor changes to human gene transfer protocols approved under Sections III–A–2 and III–B–2, in consultation with the RAC Chair and one or more RAC members, as necessary;

Section IV–C–3–d. Reviewing and approving the membership of an institution's Institutional Biosafety Committee, and where it finds the Institutional Biosafety Committee meets the requirements set forth in Section IV–B–2 will give its approval to the Institutional Biosafety Committee membership;

Section IV–C–3–e. Publishing in the **Federal Register**:

Section IV–C–3–e–(1). Announcements of RAC meetings and agendas at least 15 days in advance (Note—If the agenda for a RAC meeting is modified, ORDA shall make the revised agenda available to anyone upon request at least 72 hours in advance of the meeting);

Section IV–C–3–e–(2). Proposed Major Actions to the NIH Guidelines (see Section IV–C–1–b–(1)) at least 15 days prior to the RAC meeting;

Section IV–C–3–f. Serve as the focal point for data management of NIH-approved human gene transfer protocols approved under Sections III–A–2 and III–B–2 and registered with NIH/ORDA as required under Section III–C–7;

Section IV–C–3–g. Serve as the executive secretary of the RAC; and

Section IV–C–3–h. Maintain a list of Major and Minor Actions approved under Section III–A–2 and III–B–3 and a list of experiments registered with NIH/ORDA as described in Section III–C–7.

E. Amendment and Addition to Section V, Footnotes and References of Sections I–IV of the NIH Guidelines

The amended version of Section V–U reads as follows:

Section V–U. Human studies in which the induction or enhancement of an immune response to a vector-encoded microbial immunogen is the major goal, such an immune response has been demonstrated in model systems, and the persistence of the vector-encoded immunogen is not expected, are not covered under Sections III–A–2, III–B–2, or III–B–3. Such studies may be initiated without RAC review and NIH approval if approved by another Federal agency."

The following new footnote, V–W is added to Section V:

Section V–W. In accordance with accepted scientific and regulatory practices of the discipline of plant pathology, an exotic plant pathogen (e.g., virus, bacteria, or fungus) is one that is unknown to occur within the U.S. (see Section V–R). Determination of whether a pathogen has a potential for serious detrimental impact on managed (agricultural, forest, grassland) or natural ecosystems should be made by the Principal Investigator and the Institutional Biosafety Committee, in consultation with scientists knowledgeable of plant diseases, crops, and ecosystems in the geographic area of the research.

F. Addition to Appendix C–I, Recombinant DNA in Tissue Culture, of the NIH Guidelines

The amended version of Appendix C–I reads as follows:

Appendix C–I. Recombinant DNA in Tissue Culture

Recombinant DNA molecules containing less than one-half of any eukaryotic viral genome (all viruses from a single family (see Appendix C–VI–D) being considered identical (see Appendix C–VI–E), that are propagated and maintained in cells in tissue culture are exempt from these NIH Guidelines with the exceptions listed in Appendix C–I–A.

Appendix C–I–A. Exceptions

The following categories are not exempt from the NIH Guidelines: (i) Experiments described in Section III–A which require specific RAC review and NIH and Institutional Biosafety Committee approval before initiation, (ii) experiments described in Section III–B which require NIH/ORDA and Institutional Biosafety Committee approval before initiation, (iii) experiments involving DNA from Class 3, 4, or 5 organisms (see Appendix C–VI–A) or cells known to be infected with these agents, (iv) experiments involving the deliberate introduction of genes coding for the biosynthesis of molecules that are toxic for vertebrates (see Appendix F), and (v) whole plants regenerated from plant cells and tissue cultures are covered by the exemption provided they remain axenic cultures even though they differentiate into embryonic tissue and regenerate into plantlets.

G. Addition to Appendix G, Physical Containment, of the NIH Guidelines

Appendix G through G–I is amended to read as follows:

Appendix G specifies physical containment for standard laboratory experiments and defines Biosafety Level

1 through Biosafety Level 4. For large scale (over 10 liters) research or production, Appendix K supersedes Appendix G. Appendix K defines Good Large Scale Practice through Biosafety Level 3—Large Scale. For certain work with plants, Appendix P supersedes Appendix G. Appendix P defines Biosafety Levels 1 through 4—Plants. For certain work with animals, Appendix Q supersedes Appendix G. Appendix Q defines Biosafety Levels 1 through 4—Animals.

Appendix G–I. Standard Practices and Training

The first principle of containment is strict adherence to good microbiological practices (see Appendices G–III–A through G–III–J). Consequently, all personnel directly or indirectly involved in experiments using recombinant DNA shall receive adequate instruction (see Sections IV–B–1–e and IV–B–4–d). At a minimum, these instructions include training 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 that are known or potential biohazards shall have an emergency plan that describes the procedures to be followed if an accident contaminates personnel or the environment. The Principal Investigator shall ensure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan (see Sections IV–B–4–d and IV–B–4–e). If a research group is working with a known pathogen for which there is an effective vaccine, the vaccine should be made available to all workers. Serological monitoring, when clearly appropriate, will be provided (see Section IV–B–1–f).

The Laboratory Safety Monograph (see Appendix G–III–O) and Biosafety in Microbiological and Biomedical Laboratories (see Appendix G–III–B) describe practices, equipment, and facilities in detail.

H. Addition of Appendix P, Physical and Biological Containment for Recombinant DNA Research Involving Plants, to the NIH Guidelines

The following new appendix, Appendix P, reads as follows:

Appendix P specifies physical and biological containment conditions and practices suitable to the greenhouse conduct of experiments involving recombinant DNA-containing plants, plant-associated microorganisms, and small animals. All provisions of the NIH Guidelines apply to plant research activities with the following modifications:

Appendix P shall supersede Appendix G when the research plants are of a size, number, or have growth requirements that preclude the use of containment conditions described in Appendix G. The plants covered in Appendix P include but are not limited to mosses, liverworts, macroscopic algae, and vascular plants including terrestrial crops, forest, and ornamental species.

Plant-associated microorganisms include viroids, virusoids, viruses, bacteria, fungi, protozoans, certain small algae, and microorganisms that have a benign or beneficial association with plants, such as certain *Rhizobium* species and microorganisms known to cause plant diseases. The appendix applies to microorganisms which are being modified with the objective of fostering an association with plants.

Plant-associated small animals include those arthropods that: (i) Are in obligate association with plants, (ii) are plant pests, (iii) are plant pollinators, or (iv) transmit plant disease agents, as well as other small animals such as nematodes for which tests of biological properties necessitate the use of plants. Microorganisms associated with such small animals (e.g., pathogens or symbionts) are included.

The Institutional Biosafety Committee shall include at least one individual with expertise in plant, plant pathogen, or plant pest containment principles when experiments utilizing Appendix P require prior approval by the Institutional Biosafety Committee.

Appendix P–I. General Plant Biosafety Levels

Appendix P–I–A. The principal purpose of plant containment is to avoid the unintentional transmission of a recombinant DNA-containing plant genome, including nuclear or organelle hereditary material or release of recombinant DNA-derived organisms associated with plants.

Appendix P–I–B. The containment principles are based on the recognition that the organisms that are used pose no health threat to humans or higher animals (unless deliberately modified for that purpose), and that the containment conditions minimize the possibility of an unanticipated deleterious effect on organisms and ecosystems outside of the experimental facility, e.g., the inadvertent spread of a serious pathogen from a greenhouse to a local agricultural crop or the unintentional introduction and establishment of an organism in a new ecosystem.

Appendix P–I–C. Four biosafety levels, referred to as Biosafety Level (BL) 1—Plants (P), BL2–P, BL3–P, and BL4–P, are established in Section II. The selection of containment levels required for research involving recombinant DNA molecules in plants or associated with plants is specified in Section III. These biosafety levels are described in Appendix P–II. This appendix describes greenhouse practices and special greenhouse facilities for physical containment.

Appendix P–I–D. BL1–P through BL4–P are designed to provide differential levels of biosafety for plants in the absence or presence of other experimental organisms that contain recombinant DNA. These biosafety levels, in conjunction with biological containment conditions described in Appendix P–III, provide flexible approaches to ensure the safe conduct of research.

Appendix P–I–E. For experiments in which plants are grown at the BL1 through BL4 laboratory settings, containment practices shall be followed as described in Appendix G. These containment practices include the use of plant tissue culture rooms, growth chambers within laboratory facilities, or experiments performed on open benches. Additional biological containment practices should be added by the Greenhouse Director or Institutional Biosafety Committee as necessary (see Appendix P–III), if botanical reproductive structures are produced that have the potential of being released.

Appendix P–II. Physical Containment Levels

Appendix P–II–A. Biosafety Level 1—Plants (BL1–P)

Appendix P–II–A–1. Standard Practices (BL1–P)

Appendix P–II–A–1–a. Greenhouse Access (BL1–P)

Appendix P–II–A–1–a–(1). Access to the greenhouse shall be limited or restricted, at the discretion of the Greenhouse Director, when experiments are in progress.

Appendix P–II–A–1–a–(2). Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL1–P greenhouse practices and procedures. All procedures shall be performed in accordance with accepted greenhouse practices that are appropriate to the experimental organism.

Appendix P-II-A–1-b. Records (BL1–P)

Appendix P-II-A–1-b-(1). A record shall be kept of experiments currently in progress in the greenhouse facility.

Appendix P-II-A–1-c. Decontamination and Inactivation (BL1–P)

Appendix P-II-A–1-c-(1). Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.

Appendix P-II-A–1-d. Control of Undesired Species and Motile Macroorganisms (BL1–P)

Appendix P-II-A–1-d-(1). A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens), by methods appropriate to the organisms and in accordance with applicable state and Federal laws.

Appendix P-II-A–1-d-(2). Arthropods and other motile macroorganisms shall be housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility.

Appendix P-II-A–1-e. Concurrent Experiments Conducted in the Greenhouse (BL1–P)

Appendix P-II-A–1-e-(1). Experiments involving other organisms that require a containment level lower than BL1–P may be conducted in the greenhouse concurrently with experiments that require BL1–P containment, provided that all work is conducted in accordance with BL1–P greenhouse practices.

Appendix P-II-A–2. Facilities (BL1–P)

Appendix P-II-A–2-a. Definitions (BL1–P)

Appendix P-II-A–2-a-(1). The term 'greenhouse' refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.

Appendix P-II-A–2-a-(2). The term 'greenhouse facility' includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas, and is considered part of the confinement area.

Appendix P-II-A–2-b. Greenhouse Design (BL1–P)

Appendix P-II-A–2-b-(1). The greenhouse floor may be composed of gravel or other porous material. At a minimum, impervious (e.g., concrete) walkways are recommended.

Appendix P-II-A–2-b-(2). Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to contain or exclude pollen, microorganisms, or small flying animals (e.g., arthropods and birds); however, screens are recommended.

Appendix P-II-B. Biosafety Level 2—Plants (BL2–P)

Appendix P-II-B–1. Standard Practices (BL2–P)

Appendix P-II-B–1-a. Greenhouse Access (BL2–P)

Appendix P-II-B–1-a-(1). Access to the greenhouse shall be limited or restricted, at the discretion of the Greenhouse Director, to individuals directly involved with the experiments when they are in progress.

Appendix P-II-B–1-a-(2). Personnel shall be required to read and follow instructions on BL2–P practices and procedures. All procedures shall be conducted in accordance with accepted greenhouse practices that are appropriate to the experimental organisms.

Appendix P-II-B–1-b. Records (BL2–P)

Appendix P-II-B–1-b-(1). A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility.

Appendix P-II-B–1-b-(2). A record shall be kept of experiments currently in progress in the greenhouse facility.

Appendix P-II-B–1-b-(3). The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Greenhouse Director, Institutional Biosafety Committee, NIH/ORDA and other appropriate authorities immediately (if applicable). Reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838. Documentation of any such accident shall be prepared and maintained.

Appendix P-II-B–1-c. Decontamination and Inactivation (BL2–P)

Appendix P-II-B–1-c-(1). Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.

Appendix P-II-B–1-c-(2). Decontamination of run-off water is not necessarily required. If part of the greenhouse is composed of gravel or similar material, appropriate treatments should be made periodically to eliminate, or render inactive, any organisms potentially entrapped by the gravel.

Appendix P-II-B–1-d. Control of Undesired Species and Motile Macroorganisms (BL2–P)

Appendix P-II-B–1-d-(1). A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens) by methods appropriate to the organisms and in accordance with applicable state and Federal laws.

Appendix P-II-B–1-d-(2). Arthropods and other motile macroorganisms shall be housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility.

Appendix P-II-B–1-e. Concurrent Experiments Conducted in the Greenhouse (BL2–P)

Appendix P-II-B–1-e-(1). Experiments involving other organisms that require a containment level lower than BL2–P may be conducted in the greenhouse concurrently with experiments that require BL2–P containment provided that all work is conducted in accordance with BL2–P greenhouse practices.

Appendix P-II-B–1-f. Signs (BL2–P)

Appendix P-II-B–1-f-(1). A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) the name of the responsible individual, (ii) The plants in use, and (iii) any special requirements for using the area.

Appendix P-II-B–1-f-(2). If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence shall be indicated on a sign posted on the greenhouse access doors.

Appendix P-II-B–1-f-(3). If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.

Appendix P-II-B–1-g. Transfer of Materials (BL2–P)

Appendix P-II-B–1-g-(1). Materials containing experimental microorganisms, which are brought into or removed from the greenhouse facility in a viable or intact state, shall be transferred in a closed non-breakable container.

Appendix P-II-B–1-h. Greenhouse Practices Manual (BL2–P)

Appendix P-II-B–1-h-(1). A greenhouse practices manual shall be

prepared or adopted. This manual shall: (i) Advise personnel of the potential consequences if such practices are not followed, and (ii) outline contingency plans to be implemented in the event of the unintentional release of organisms.

Appendix P-II-B–2. Facilities (BL2–P)

Appendix P-II-B–2-a. Definitions (BL2–P)

Appendix P-II-B–2-a-(1). The term 'greenhouse' refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.

Appendix P-II-B–2-a-(2). The term 'greenhouse facility' includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas and is considered part of the confinement area.

Appendix P-II-B–2-b. Greenhouse Design (BL2–P)

Appendix P-II-B–2-b-(1). A greenhouse floor composed of an impervious material. Concrete is recommended, but gravel or other porous material under benches is acceptable unless propagules of experimental organisms are readily disseminated through soil. Soil beds are acceptable unless propagules of experimental organisms are readily disseminated through soil.

Appendix P-II-B–2-b-(2). Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to exclude pollen or microorganisms; however, screens are required to exclude small flying animals (e.g., arthropods and birds).

Appendix P-II-B–2-c. Autoclaves (BL2–P)

Appendix P-II-B–2-c-(1). An autoclave shall be available for the treatment of contaminated greenhouse materials.

Appendix P-II-B–2-d. Supply and Exhaust Air Ventilation Systems (BL2–P)

Appendix P-II-B–2-d-(1). If intake fans are used, measures shall be taken to minimize the ingress of arthropods. Louvers or fans shall be constructed such that they can only be opened when the fan is in operation.

Appendix P-II-B–2-e. Other (BL2–P)

Appendix P-II-B–2-e-(1). BL2–P greenhouse containment requirements may be satisfied by using a growth chamber or growth room within a building provided that the external physical structure limits access and escape of microorganisms and macroorganisms in a manner that satisfies the intent of the foregoing clauses.

Appendix P-II-C. Biosafety Level 3—Plants (BL3–P)

Appendix P-II-C–1. Standard Practices (BL3–P)

Appendix P-II-C–1-a. Greenhouse Access (BL3–P)

Appendix P-II-C–1-a-(1). Authorized entry into the greenhouse shall be restricted to individuals who are required for program or support purposes. The Greenhouse Director shall be responsible for assessing each circumstance and determining those individuals who are authorized to enter the greenhouse facility.

Appendix P-II-C–1-a-(2). Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL3–P practices and procedures. All procedures shall be conducted in accordance with accepted greenhouse practices that are appropriate to the experimental organisms.

Appendix P-II-C–1-b. Records (BL3–P)

Appendix P-II-C–1-b-(1). A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility.

Appendix P-II-C–1-b-(2). A record shall be kept of experiments currently in progress in the greenhouse facility.

Appendix P-II-C–1-b-(3). The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Biological Safety Officer, Greenhouse Director, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities immediately (if applicable).

Reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838. Documentation of any such accident shall be prepared and maintained.

Appendix P-II-C–1-c. Decontamination and Inactivation (BL3–P)

Appendix P-II-C–1-c-(1). All experimental materials shall be sterilized in an autoclave or rendered biologically inactive by appropriate methods before disposal, except those that are to remain in a viable or intact state for experimental purposes; including water that comes in contact with experimental microorganisms or with material exposed to such microorganisms, and contaminated equipment and supplies.

Appendix P-II-C–1-d. Control of Undesired Species and Motile Macroorganisms (BL3–P)

Appendix P-II-C–1-d-(1). A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens) by methods appropriate to the organisms and in accordance with applicable state and Federal laws.

Appendix P-II-C–1-d-(2). Arthropods and other motile macroorganisms shall be housed in appropriate cages. When appropriate to the organism, experiments shall be conducted within cages designed to contain the motile organisms.

Appendix P-II-C–1-e. Concurrent Experiments Conducted in the Greenhouse (BL3–P)

Appendix P-II-C–1-e-(1). Experiments involving organisms that require a containment level lower than BL3–P may be conducted in the greenhouse concurrently with experiments that require BL3–P containment provided that all work is conducted in accordance with BL3–P greenhouse practices.

Appendix P-II-C–1-f. Signs (BL3–P)

Appendix P-II-C–1-f-(1). A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) The name of the responsible individual, (ii) the plants in use, and (iii) any special requirements for using the area.

Appendix P-II-C–1-f-(2). If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence should be indicated on a sign posted on the greenhouse access doors.

Appendix P-II-C–1-f-(3). If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.

Appendix P-II-C–1-g. Transfer of Materials (BL3–P)

Appendix P-II-C–1-g-(1). Experimental materials that are brought into or removed from the greenhouse facility in a viable or intact state shall be transferred to a non-breakable sealed secondary container. At the time of transfer, if the same plant species, host, or vector are present within the effective dissemination distance of propagules of the experimental organism, the surface

of the secondary container shall be decontaminated. Decontamination may be accomplished by passage through a chemical disinfectant or fumigation chamber or by an alternative procedure that has demonstrated effective inactivation of the experimental organism.

Appendix P-II-C–1-h. Greenhouse Practices Manual (BL3–P)

Appendix P-II-C–1-h-(1). A greenhouse practices manual shall be prepared or adopted. This manual shall: (i) Advise personnel of the potential consequences if such practices are not followed, and (ii) outline contingency plans to be implemented in the event of the unintentional release of organisms with recognized potential for serious detrimental impact.

Appendix P-II-C–1-i. Protective Clothing (BL3–P)

Appendix P-II-C–1-i-(1). Disposable clothing (e.g., solid front or wrap-around gowns, scrub suits, or other appropriate clothing) shall be worn in the greenhouse if deemed necessary by the Greenhouse Director because of potential dissemination of the experimental microorganisms.

Appendix P-II-C–1-i-(2). Protective clothing shall be removed before exiting the greenhouse and decontaminated prior to laundering or disposal.

Appendix P-II-C–1-j. Other (BL3–P)

Appendix P-II-C–1-j-(1). Personnel are required to thoroughly wash their hands upon exiting the greenhouse.

Appendix P-II-C–1-j-(2). All procedures shall be performed carefully to minimize the creation of aerosols and excessive splashing of potting material/soil during watering, transplanting, and all experimental manipulations.

Appendix P-II-C–2. Facilities (BL3–P)

Appendix P-II-C–2-a. Definitions (BL3–P)

Appendix P-II-C–2-a-(1). The term 'greenhouse' refers to a structure with walls, roof, and floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.

Appendix P-II-C–2-a-(2). The term 'greenhouse facility' includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas, and is considered part of the confinement area. The need to maintain negative pressure should be considered when constructing or renovating the greenhouse.

Appendix P-II-C–2-b. Greenhouse Design (BL3–P)

Appendix P-II-C–2-b-(1). The greenhouse floor shall be composed of concrete or other impervious material with provision for collection and decontamination of liquid run-off.

Appendix P-II-C–2-b-(2). Windows shall be closed and sealed. All glazing shall be resistant to breakage (e.g., double-pane tempered glass or equivalent).

Appendix P-II-C–2-b-(3). The greenhouse shall be a closed self-contained structure with a continuous covering that is separated from areas that are open to unrestricted traffic flow. The minimum requirement for greenhouse entry shall be passage through two sets of self-closing locking doors.

Appendix P-II-C–2-b-(4). The greenhouse facility shall be surrounded by a security fence or protected by equivalent security measures.

Appendix P-II-C–2-b-(5). Internal walls, ceilings, and floors shall be resistant to penetration by liquids and chemicals to facilitate cleaning and decontamination of the area. All penetrations into these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

Appendix P-II-C–2-b-(6). Bench tops and other work surfaces should have seamless surfaces that are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix P-II-C–2-b-(7). The greenhouse contains a foot, elbow, or automatically operated sink, which is located near the exit door for hand washing.

Appendix P-II-C–2-c. Autoclaves (BL3–P)

Appendix P-II-C–2-c-(1). An autoclave shall be available for decontaminating materials within the greenhouse facility. A double-door autoclave is recommended (not required) for the decontamination of materials passing out of the greenhouse facility.

Appendix P-II-C–2-d. Supply and Exhaust Air Ventilation Systems (BL3–P)

Appendix P-II-C–2-d-(1). An individual supply and exhaust air ventilation system shall be provided. The system maintains pressure differentials and directional airflow, as required, to assure inward (or zero) airflow from areas outside of the greenhouse.

Appendix P-II-C–2-d-(2). The exhaust air from the greenhouse facility shall be filtered through high efficiency particulate air-HEPA filters and discharged to the outside. The filter chambers shall be designed to allow *in situ* decontamination before filters are removed and to facilitate certification testing after they are replaced. Air filters shall be 80–85% average efficiency by the American Society of Heating, Refrigerating, and Air Conditioning Engineers (ASHRAE) Standard 52-68 test method using atmosphere dust. Air supply fans shall be equipped with a back-flow damper that closes when the air supply fan is off. Alternatively, a HEPA filter may be used on the air supply system instead of the filters and damper. The supply and exhaust airflow shall be interlocked to assure inward (or zero) airflow at all times.

Appendix P–II–C–2–e. Other (BL3–P)

Appendix P–II–C–2–e–(1). BL3–P greenhouse containment requirements may be satisfied using a growth chamber or growth room within a building provided that the location, access, airflow patterns, and provisions for decontamination of experimental materials and supplies meet the intent of the foregoing clauses.

Appendix P–II–C–2–e–(2). Vacuum lines shall be protected with high efficiency particulate air/HEPA or equivalent filters and liquid disinfectant traps.

Appendix P–II–D. Biosafety Level 4—Plants (BL4–P)

Appendix P–II–D–1. Standard Practices (BL4–P)

Appendix P–II–D–1–a. Greenhouse Access (BL4–P)

Appendix P–II–D–1–a–(1). Authorized entry into the greenhouse shall be restricted to individuals who are required for program or support purposes. The Greenhouse Director shall be responsible for assessing each circumstance and determining those individuals who are authorized to enter the greenhouse facility or work in the greenhouse during experiments.

Appendix P–II–D–1–a–(2). Access shall be managed by the Greenhouse Director, Biological Safety Officer, or other individual responsible for physical security of the greenhouse facility; and access limited by means of secure, locked doors.

Appendix P–II–D–1–a–(3). Prior to entering, individuals shall be advised of the potential environmental hazards and instructed on appropriate safeguards for ensuring environmental safety. Individuals authorized to enter the

greenhouse facility shall comply with the instructions and all other applicable entry/exit procedures.

Appendix P–II–D–1–a–(4). Personnel shall enter and exit the greenhouse facility only through the clothing change and shower rooms and shall shower each time they exit the greenhouse facility. Personnel shall use the airlocks to enter or exit the laboratory only in an emergency. In the event of an emergency, every reasonable effort should be made to prevent the possible transport of viable propagules from containment.

Appendix P–II–D–1–a–(5). Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL4–P practices and procedures.

Appendix P–II–D–1–b. Records (BL4–P)

Appendix P–II–D–1–b–(1). A record shall be kept of all experimental materials brought into or removed from the greenhouse.

Appendix P–II–D–1–b–(2). A record shall be kept of experiments currently in progress in the greenhouse facility.

Appendix P–II–D–1–b–(3). A record shall be kept of all personnel entering and exiting the greenhouse facility, including the date and time of each entry.

Appendix P–II–D–1–b–(4). The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Biological Safety Officer, Greenhouse Director, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities immediately (if applicable). Reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838. Documentation of any such accident shall be prepared and maintained.

Appendix P–II–D–1–c. Decontamination and Inactivation (BL4–P)

Appendix P–II–D–1–c–(1). All materials, except for those that are to remain in a viable or intact state for experimental purposes, shall be autoclaved prior to removal from the maximum containment greenhouse. Equipment or material that could be damaged by high temperatures or steam shall be decontaminated by alternative methods (e.g., gas or vapor sterilization) in an airlock or chamber designed for this purpose.

Appendix P–II–D–1–c–(2). Water that comes in contact with experimental microorganisms or with material exposed to such microorganisms (e.g., run-off from watering plants) shall be collected and decontaminated before disposal.

Appendix P–II–D–1–c–(3). Standard microbiological procedures shall be followed for decontamination of equipment and materials. Spray or liquid waste or rinse water from containers used to apply the experimental microorganisms shall be decontaminated before disposal.

Appendix P–II–D–1–d. Control of Undesired Species and Motile Macroorganisms (BL4–P)

Appendix P–II–D–1–d–(1). A chemical control program shall be implemented to eliminate undesired pests and pathogens in accordance with applicable state and Federal laws.

Appendix P–II–D–1–d–(2). Arthropods and other motile macroorganisms used in conjunction with experiments requiring BL4–P level physical containment shall be housed in appropriate cages. When appropriate to the organism, experiments shall be conducted within cages designed to contain the motile organisms.

Appendix P–II–D–1–e. Concurrent Experiments Conducted in the Greenhouse (BL4–P)

Appendix P–II–D–1–e–(1). Experiments involving organisms that require a containment level lower than BL4–P may be conducted in the greenhouse concurrently with experiments that require BL4–P containment provided that all work is conducted in accordance with BL4–P greenhouse practices. When the experimental microorganisms in use require a containment level lower than BL4–P, greenhouse practices reflect the level of containment required by the highest containment level microorganisms being tested.

Appendix P–II–D–1–f. Signs (BL4–P)

Appendix P–II–D–1–f–(1). A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) The name of the responsible individual, (ii) the plants in use, and (iii) any special requirements for using the area.

Appendix P–II–D–1–f–(2). If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence shall be indicated by a sign posted on the greenhouse access doors.

Appendix P–II–D–1–f–(3). If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.

Appendix P–II–D–1–g. Transfer of Materials (BL4–P)

Appendix P–II–D–1–g–(1). Experimental materials that are brought into or removed from the greenhouse in a viable or intact state shall be transferred to a non-breakable, sealed, primary container then enclosed in a non-breakable, sealed secondary container. These containers shall be removed from the greenhouse facility through a chemical disinfectant, fumigation chamber, or an airlock designed for this purpose.

Appendix P–II–D–g–(2). Supplies and materials shall be brought into the greenhouse facility through a double-door autoclave, fumigation chamber, or airlock that is appropriately decontaminated between each use. After securing the outer doors, personnel within the greenhouse facility shall retrieve the materials by opening the interior door of the autoclave, fumigation chamber, or airlock. These doors shall be secured after the materials are brought into the greenhouse facility.

Appendix P–II–D–1–h. Greenhouse Practices Manual (BL4–P)

Appendix P–II–D–1–h–(1). A greenhouse practices manual shall be prepared or adopted. This manual shall include contingency plans to be implemented in the event of the unintentional release of experimental organisms.

Appendix P–II–D–1–i. Protective Clothing (BL4–P)

Appendix P–II–D–1–i–(1). Street clothing shall be removed in the outer clothing change room. Complete laboratory clothing (may be disposable) including undergarments, pants, and shirts, jump suits, shoes, and hats shall be provided and worn by all personnel entering the greenhouse facility.

Appendix P–II–D–1–i–(2). Personnel shall remove laboratory clothing when exiting the greenhouse facility and before entering the shower area. This clothing shall be stored in a locker or hamper in the inner change room.

Appendix P–II–D–1–i–(3). All laboratory clothing shall be autoclaved before laundering.

Appendix P–II–D–2. Facilities (BL4–P)

Appendix P–II–D–2–a. Greenhouse Design (BL4–P)

Appendix P–II–D–2–a–(1). The maximum containment greenhouse facility shall consist of a separate building or a clearly demarcated and isolated area within a building. The need to maintain negative pressure

should be considered when constructing or renovating the greenhouse facility.

Appendix P–II–D–2–a–(2). Outer and inner change rooms, separated by a shower, shall be provided for personnel entering and exiting the greenhouse facility.

Appendix P–II–D–2–a–(3). Windows shall be closed and sealed. All glazing shall be resistant to breakage (e.g., double-pane tempered glass or equivalent).

Appendix P–II–D–2–a–(4). Access doors to the greenhouse shall be self-closing and locking.

Appendix P–II–D–2–a–(5). The greenhouse facility shall be surrounded by a security fence or protected by equivalent security measures.

Appendix P–II–D–2–a–(6). The walls, floors, and ceilings of the greenhouse shall be constructed to form a sealed internal shell that facilitates fumigation and is animal and arthropod-proof. These internal surfaces shall be resistant to penetration and degradation by liquids and chemicals to facilitate cleaning and decontamination of the area. All penetrations into these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

Appendix P–II–D–2–a–(7). Bench tops and other work surfaces shall have seamless surfaces impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix P–II–D–2–a–(8). A double-door autoclave, fumigation chamber, or ventilated airlock shall be provided for passage of all materials, supplies, or equipment that are not brought into the greenhouse facility through the change room.

Appendix P–II–D–2–b. Autoclaves (BL4–P)

Appendix P–II–D–2–b–(1). A double-door autoclave shall be provided for the decontamination of materials removed from the greenhouse facility. The autoclave door, which opens to the area external to the greenhouse facility, shall be sealed to the outer wall and automatically controlled so that it can only be opened upon completion of the sterilization cycle.

Appendix P–II–D–2–c. Supply and Exhaust Air Ventilation Systems (BL4–P)

Appendix P–II–D–2–c–(1). An individual supply and exhaust air ventilation system shall be provided. The system shall maintain pressure differentials and directional airflow as required to assure inward (or zero) airflow from areas outside of the greenhouse. Differential pressure transducers shall be used to sense pressure levels. If a system malfunctions, the transducers shall sound an alarm. A backup source of power should be considered. The supply and exhaust airflow shall be interlocked to assure inward (or zero) airflow at all times. The integrity of the greenhouse shall have an air leak rate (decay rate) not to exceed 7 percent per minute (logarithm of pressure against time) over a 20-minute period at 2 inches of water gauge pressure. Nominally, this is 0.05 inches of water gauge pressure loss in 1 minute at 2 inches water gauge pressure.

Appendix P–II–D–2–c–(2). Exhaust air from the greenhouse facility shall be filtered through high efficiency particulate air/HEPA filters and discharged to the outside and dispersed away from occupied buildings and air intakes. Filter chambers shall be designed to allow *in situ* decontamination before filters are removed and to facilitate certification testing after they are replaced. HEPA filters shall be provided to treat air supplied to the greenhouse facility. HEPA filters shall be certified annually.

Appendix P–II–D–2–d. Other (BL4–P)

Appendix P–II–D–2–d–(1). Sewer vents and other ventilation lines contain high efficiency particulate air/HEPA filters. HEPA filters shall be certified annually.

Appendix P–II–D–2–d–(2). A pass-through dunk tank, fumigation chamber, or an equivalent method of decontamination shall be provided to ensure decontamination of materials and equipment that cannot be decontaminated in the autoclave.

Appendix P–II–D–2–d–(3). Liquid effluent from sinks, floors, and autoclave chambers shall be decontaminated by heat or chemical treatment before being released from the maximum containment greenhouse facility. Liquid wastes from shower rooms and toilets may be decontaminated by heat or chemical treatment. Autoclave and chemical decontamination of liquid wastes shall be evaluated by appropriate standard procedures for autoclaved wastes. Decontamination shall be evaluated mechanically and biologically using a recording thermometer and an indicator microorganism with a defined heat susceptibility pattern. If liquid wastes are decontaminated with chemical disinfectants, the chemicals used must have demonstrated efficacy against the target or indicator microorganisms.

Appendix P–II–D–2–d–(4). If there is a central vacuum system, it shall not serve areas outside of the greenhouse facility. In-line high efficiency particulate air/HEPA filters shall be placed as near as practicable to each use point or vacuum service cock. Other liquid and gas services to the greenhouse facility shall be protected by devices that prevent back-flow. HEPA filters shall be certified annually.

Appendix P–III. Biological Containment Practices

Appropriate selection of the following biological containment practices may be used to meet the containment requirements for a given organism. The present list is not exhaustive; there may be other ways of preventing effective dissemination that could possibly lead to the establishment of the organism or its genetic material in the environment resulting in deleterious consequences to managed or natural ecosystems.

Appendix P–III–A. Biological Containment Practices (Plants)

Appendix P–III–A–1. Effective dissemination of plants by pollen or seed can be prevented by one or more of the following procedures: (i) Cover the reproductive structures to prevent pollen dissemination at flowering and seed dissemination at maturity; (ii) remove reproductive structures by employing male sterile strains, or harvest the plant material prior to the reproductive stage; (iii) ensure that experimental plants flower at a time of year when cross-fertile plants are not flowering within the normal pollen dispersal range of the experimental plant; or (iv) ensure that cross-fertile plants are not growing within the known pollen dispersal range of the experimental plant.

Appendix P-III-B. Biological Containment Practices (Microorganisms)

Appendix P-III-B–1. Effective dissemination of microorganisms beyond the confines of the greenhouse can be prevented by one or more of the following procedures: (i) Confine all operations to injections of microorganisms or other biological procedures (including genetic manipulation) that limit replication or reproduction of viruses and microorganisms or sequences derived from microorganisms, and confine these injections to internal plant parts or adherent plant surfaces; (ii) ensure that organisms, which can serve as hosts or promote the transmission of the virus or microorganism, are not present within the farthest distance that the airborne virus or microorganism may be expected to be effectively disseminated; (iii) conduct experiments at a time of year when plants that can serve as hosts are either not growing or are not susceptible

to productive infection; (iv) use viruses and other microorganisms or their genomes that have known arthropod or animal vectors, in the absence of such vectors; (v) use microorganisms that have an obligate association with the plant; or (vi) use microorganisms that are genetically disabled to minimize survival outside of the research facility and whose natural mode of transmission requires injury of the target organism, or assures that inadvertent release is unlikely to initiate productive infection of organisms outside of the experimental facility.

Appendix P-III-C. Biological Containment Practices (Macroorganisms)

Appendix P-III-C–1. Effective dissemination of arthropods and other small animals can be prevented by using one or more of the following procedures: (i) Use non-flying, flight-impaired, or sterile arthropods; (ii) use non-motile or sterile strains of small animals; (iii) conduct experiments at a time of year that precludes the survival of escaping organisms; (iv) use animals that have an obligate association with a plant that is not present within the dispersal range of the organism; or (v) prevent the escape of organisms present in run-off water by chemical treatment or evaporation of run-off water.

I. Addition of Appendix Q, Physical and Biological Containment for Recombinant DNA Research Involving Animals, to the NIH Guidelines

The following new appendix, Appendix Q, reads as follows:

Appendix Q specifies containment and confinement practices for research involving whole animals, both those in which the animal's genome has been altered by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant DNA-modified microorganisms tested on whole animals. The appendix applies to animal research activities with the following modifications:

Appendix Q shall supersede Appendix G when research animals are of a size or have growth requirements that preclude the use of containment for laboratory animals. Some animals may require other types of containment (see Appendix Q-III-D). The animals covered in Appendix Q are those species normally categorized as animals including but not limited to cattle, swine, sheep, goats, horses, and poultry.

The Institutional Biosafety Committee shall include at least one scientist with expertise in animal containment principles when experiments utilizing Appendix Q require Institutional Biosafety Committee prior approval.

The institution shall establish and maintain a health surveillance program for personnel engaged in animal research involving viable recombinant DNA-containing microorganisms that require Biosafety Level (BL) 3 or greater containment in the laboratory.

Appendix Q-I. General Considerations

Appendix Q-I-A. Containment Levels

The containment levels required for research involving recombinant DNA associated with or in animals is based on classification of experiments in Section III. For the purpose of animal research, four levels of containment are established. These are referred to as BL1–Animals (N), BL2–N, BL3–N, and BL4–N and are described in the following sections of Appendix Q. The descriptions include: (i) standard practices for physical and biological containment, and (ii) animal facilities.

Appendix Q-I-B. Disposal of Animals (BL1–N through BL4–N)

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

Appendix Q-I-B–2. A permanent record shall be maintained of the experimental use and disposal of each animal or group of animals.

Appendix Q-II. Physical and Biological Containment Levels

Appendix Q-II-A. Biosafety Level 1 - Animals (BL1–N)

Appendix Q-II-A–1. Standard Practices (BL1–N)

Appendix Q-II-A–1-a. Animal Facility Access (BL1–N)

Appendix Q-II-A–1-a-(1). The containment area shall be locked.

Appendix Q-II-A–1-a-(2). Access to the containment area shall be limited or restricted when experimental animals are being held.

Appendix Q-II-A–1-a-(3). The containment area shall be patrolled or monitored at frequent intervals.

Appendix Q-II-A–1-b. Other (BL1–N)

Appendix Q-II-A–1-b-(1). All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non- transgenic animals.

Appendix Q-II-A–1-b-(2) A double barrier shall be provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used.

Appendix Q-II-A–1-b-(3). The containment area shall be in accordance with state and Federal laws and animal care requirements.

Appendix Q-II-A–2. Animal Facilities (BL1–N)

Appendix Q-II-A–2-(a). Animals shall be confined to securely fenced areas or be in enclosed structures (animal rooms) to minimize the possibility of theft or unintentional release.

Appendix Q-II-B. Biosafety Level 2 - Animals (BL2–N) (see Appendix Q-III-A)

Appendix Q-II-B–1. Standard Practices (BL2–N)

Appendix Q-II-B–1-a. Animal Facility Access (BL2–N)

Appendix Q-II-B–1-a-(1). The containment area shall be locked.

Appendix Q-II-B–1-a-(2). The containment area shall be patrolled or monitored at frequent intervals.

Appendix Q-II-B–1-a-(3). The containment building shall be controlled and have a locking access.

Appendix Q-II-B–1-a-(4). The Animal Facility Director shall establish policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) may enter the laboratory or animal rooms.

Appendix Q-II-B–1-a-(5). Animals of the same or different species, which are not involved in the work being performed, shall not be permitted in the animal area.

Appendix Q-II-B–1-b. Decontamination and Inactivation (BL2–N)

Appendix Q-II-B–1-b-(1). Contaminated materials that are decontaminated at a site away from the laboratory shall be placed in a closed durable leak-proof container prior to removal from the laboratory.

Appendix Q-II-B–1-b-(2). Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q-II-B–1-c. Signs (BL2–N)

Appendix Q-II-B–1-c-(1). When the animal research requires special provisions for entry (e.g., vaccination), a warning sign incorporating the universal biosafety symbol shall be posted on all access doors to the animal work area. The sign shall indicate: (i) The agent, (ii) the animal species, (iii) the name and telephone number of the Animal Facility Director or other responsible individual, and (iv) any special requirements for entering the laboratory.

Appendix Q-II-B–1-d. Protective Clothing (BL2–N)

Appendix Q-II-B–1-d-(1). Laboratory coats, gowns, smocks, or uniforms shall be worn while in the animal area or attached laboratory. Before entering non-laboratory areas (e.g., cafeteria, library, administrative offices), protective clothing shall be removed and kept in the work entrance area.

Appendix Q-II-B–1-d-(2). Special care shall be taken to avoid skin contamination with microorganisms containing recombinant DNA. Impervious and/or protective gloves shall be worn when handling experimental animals and when skin contact with an infectious agent is unavoidable.

Appendix Q-II-B–1-e. Records (BL2–N)

Appendix Q-II-B–1-e-(1). Any incident involving spills and accidents that result in environmental release or exposures of animals or laboratory workers to organisms containing recombinant DNA molecules shall be reported immediately to the Animal Facility Director, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities (if applicable). Reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838. Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.

Appendix Q-II-B–1-e-(2). When appropriate and giving consideration to the agent handled, baseline serum samples shall be collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agent handled and the function of the animal facility.

Appendix Q-II-B–1-f. Transfer of Materials (BL2–N)

Appendix Q-II-B–1-f-(1). Biological materials removed from the animal containment area in a viable or intact state shall be transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container. All containers, primary and secondary, shall be disinfected before removal from the animal facility. Advance approval for transfer of material shall be obtained from the Animal Facility Director. Packages containing viable agents may only be opened in a facility having an equivalent or higher level of physical containment unless the agent is biologically inactivated or incapable of reproduction.

Appendix Q-II-B–1-g. Other (BL2–N)

Appendix Q-II-B–1-g-(1). All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

Appendix Q-II-B–1-g-(2). Needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) shall be used for the injection or aspiration of fluids containing organisms that contain recombinant DNA. Extreme caution shall be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Following use, needles shall not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe. Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q-II-B–1-g-(3). Appropriate steps should be taken to prevent horizontal transmission or exposure of laboratory personnel. If the agent used as a vector is known to be transmitted by a particular route (e.g., arthropods), special attention should be given to preventing spread by that route. In the absence of specific knowledge of a particular route of transmission, all potential means of horizontal transmission (e.g., arthropods, contaminated bedding, or animal waste, etc.) should be prevented.

Appendix Q-II-B–1-g-(4). Eating, drinking, smoking, and applying cosmetics shall not be permitted in the work area.

Appendix Q-II-B–1-g-(5). Individuals who handle materials and animals containing recombinant DNA molecules shall be required to wash their hands before exiting the containment area.

Appendix Q-II-B–1-g-(6). A double barrier shall be provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used.

Appendix Q-II-B–1-g-(7). The containment area shall be in accordance with state and Federal laws and animal care requirements.

Appendix Q-II-B–1-g-(8). A biosafety manual shall be prepared or adopted. Personnel shall be advised of special hazards and required to read and follow instructions on practices and procedures.

Appendix Q-II-B–2. Animal Facilities (BL2–N)

Appendix Q-II-B–2-a. Animals shall be contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and to avoid arthropod access. The special provision to avoid the entry or escape of arthropods from the animal areas may be waived if the agent in use is not known to be transmitted by arthropods.

Appendix Q-II-B–2-b. Surfaces shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix Q-II-B–2-c. The animal containment area shall be designed so that it can be easily cleaned.

Appendix Q-II-B–2-d. Windows that open shall be fitted with fly screens.

Appendix Q-II-B–2-e. An autoclave shall be available for decontamination of laboratory wastes.

Appendix Q-II-B–2-f. If arthropods are used in the experiment or the agent under study can be transmitted by an arthropod, interior work areas shall be appropriately screened (52 mesh). All perimeter joints and openings shall be sealed and additional arthropod control mechanisms used to minimize arthropod entry and propagation, including appropriate screening of access doors or the equivalent.

Appendix Q-II-C. Biosafety Level 3—Animals (BL3–N) (See Appendix Q-III-B)

Appendix Q-II-C–1. Standard Practices (BL3–N)

Appendix Q-II-C–1-a. Animal Facility Access (BL3–N)

Appendix Q-II-C–1-a-(1). The containment area shall be locked.

Appendix Q-II-C–1-a-(2). The containment area shall be patrolled or monitored at frequent intervals.

Appendix Q-II-C–1-a-(3). The containment building shall be controlled and have a locking access.

Appendix Q-II-C–1-a-(4). The Animal Facility Director shall establish policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) shall enter the laboratory or animal rooms.

Appendix Q-II-C–1-a-(5). Animal room doors, gates, or other closures shall be kept closed when experiments are in progress.

Appendix Q-II-C–1-b. Decontamination and Inactivation (BL3–N)

Appendix Q-II-C–1-b-(1). The work surfaces of containment equipment shall be decontaminated when work with organisms containing recombinant DNA molecules is finished. Where feasible, plastic-backed paper toweling shall be used on nonporous work surfaces to facilitate clean-up.

Appendix Q-II-C–1-b-(2). All animals shall be euthanized at the end of their experimental usefulness and the carcasses decontaminated before disposal in an approved manner.

Appendix Q-II-C–1-b-(3). Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q-II-C–1-b-(4). Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval shall be required to transfer agents or tissue/organ specimens from a BL3–N animal facility to a facility with a lower containment classification.

Appendix Q-II-C–1-b-(5). Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated every 30 days with an indicator organism.

Appendix Q-II-C–1-c. Signs (BL3–N)

Appendix Q-II-C–1-c-(1). When the animal research requires special provisions for entry (e.g., vaccination), a warning sign incorporating the universal biosafety symbol shall be posted on all access doors to the animal work area. The sign shall indicate: (i) The agent, (ii) the animal species, (iii) the name and telephone number of the Animal Facility Director or other responsible individual, and (iv) any special requirements for entering the laboratory.

Appendix Q-II-C–1-d. Protective Clothing (BL3–N)

Appendix Q-II-C–1-d-(1). Full protective clothing that protects the individual (e.g., scrub suits, coveralls, uniforms) shall be worn in the animal area. Clothing shall not be worn outside the animal containment area and shall be decontaminated before laundering or disposal. Personnel shall be required to shower before exiting the BL3–N area and wearing of personal clothing.

Appendix Q-II-C–1-d-(2). Special care shall be taken to avoid skin contamination with microorganisms containing recombinant DNA. Impervious and/or protective gloves shall be worn when handling experimental animals and when skin contact with an infectious agent is unavoidable.

Appendix Q-II-C–1-d-(3). Appropriate respiratory protection shall be worn in rooms containing experimental animals.

Appendix Q-II-C–1-e. Records (BL3–N)

Appendix Q-II-C–1-e-(1). Documents regarding experimental animal use and disposal shall be maintained in a permanent record book.

Appendix Q-II-C–1-e-(2). Any incident involving spills and accidents that result in environmental release or exposure of animals or laboratory workers to organisms containing recombinant DNA shall be reported immediately to the Biological Safety Office, Animal Facility Director, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities (if applicable). Reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838. Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.

Appendix Q-II-C–1-e-(3). When appropriate and giving consideration to the agent handled, baseline serum samples shall be collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agent handled or the function of the facility.

Appendix Q-II-C–1-f. Transfer of Materials (BL3–N)

Appendix Q-II-C–1-f-(1). Biological materials removed from the animal containment laboratory in a viable or intact state shall be transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container. All containers, primary and secondary, shall be disinfected before removal from the animal facility. Advance approval for transfer of material shall be obtained from the Animal Facility Director. Packages containing viable agents may be opened only in a facility having an equivalent or higher level of physical containment unless the agent is biologically inactivated or incapable of reproduction.

Appendix Q-II-C–1-f-(2). Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval shall be required to transfer agents or tissue/organ specimens from a BL3–N animal facility to a facility with a lower containment classification.

Appendix Q-II-C–1-g. Other (BL3–N)

Appendix Q-II-C–1-g-(1). All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among nontransgenic animals.

Appendix Q-II-C–1-g-(2). Appropriate steps should be taken to prevent horizontal transmission or exposure of laboratory personnel. If the agent used as the vector is known to be transmitted by a particular route (e.g., arthropods), special attention should be given to preventing spread by that route. In the absence of specific knowledge of a particular route of transmission, all potential means of horizontal transmission (e.g., arthropods, contaminated bedding, or animal waste) should be prevented.

Appendix Q-II-C–1-g-(3). Eating, drinking, smoking, and applying cosmetics shall not be permitted in the work area.

Appendix Q-II-C-1-g-(4). Individuals who handle materials and animals containing recombinant DNA molecules shall be required to wash their hands before exiting the containment area.

Appendix Q-II-C-1-g-(5). Experiments involving other organisms that require containment levels lower than BL3–N may be conducted in the same area concurrently with experiments requiring BL3–N containment provided that they are conducted in accordance with BL3–N practices.

Appendix Q-II-C-1-g-(6). Animal holding areas shall be cleaned at least once a day and decontaminated immediately following any spill of viable materials.

Appendix Q-II-C-1-g-(7). All procedures shall be performed carefully to minimize the creation of aerosols.

Appendix Q-II-C-1-g-(8). A double barrier shall be provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used.

Appendix Q-II-C-1-g-(9). The containment area shall be in accordance with state and Federal laws and animal care requirements.

Appendix Q-II-C-1-g-(10). All animals shall be euthanized at the end of their experimental usefulness and the carcasses decontaminated before disposal in an approved manner.

Appendix Q-II-C-1-g-(11). Personnel shall be required to shower before exiting the BL3–N area and wearing personal clothing.

Appendix Q-II-C-1-g-(12). Animals of the same or different species, which are not involved in the work being performed, shall not be permitted in the animal area.

Appendix Q-II-C-1-g-(13). Needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) shall be used for the injection or aspiration of fluids containing organisms that contain recombinant DNA. Extreme caution shall be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Following use, needles shall not be bent, sheared, replaced in the needle sheath or guard or removed from the syringe. The needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q-II-C-1-g-(14). A biosafety manual shall be prepared or adopted. Personnel shall be advised of special hazards and required to read and follow instructions on practices and procedures.

Appendix Q-II-C-2. Animal Facilities (BL3–N)

Appendix Q-II-C-2-a. Animals shall be contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and avoid arthropod access. The special provision to avoid the entry or escape of arthropods from the animal areas may be waived if the agent in use is not known to be transmitted by arthropods.

Appendix Q–II–C–2–b. The interior walls, floors, and ceilings shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat, to facilitate cleaning. Penetrations in these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

Appendix Q–II–C–2–c. Windows in the animal facility shall be closed, sealed, and breakage resistant (e.g., double-pane tempered glass or equivalent). The need to maintain negative pressure should be considered when constructing or renovating the animal facility.

Appendix Q–II–C–2–d. An autoclave, incinerator, or other effective means to decontaminate animals and waste shall be available, preferably within the containment area. If feasible, a double-door autoclave is preferred and should be positioned to allow removal of material from the containment area.

Appendix Q–II–C–2–e. If arthropods are used in the experiment or the agent under study can be transmitted by an arthropod, the interior work area shall be appropriately screened (52 mesh). All perimeter joints and openings shall be sealed, and additional arthropod control mechanisms used to minimize arthropod entry and propagation, including appropriate screening, or the equivalent of access doors.

Appendix Q–II–C–2–f. Access doors to the containment area shall be self-closing.

Appendix Q–II–C–2–g. The animal area shall be separated from all other areas. Passage through two sets of doors shall be the basic requirement for entry into the animal area from access corridors or other contiguous areas. The animal containment area shall be physically separated from access corridors and other laboratories or areas by a double-door clothes change room, equipped with integral showers and airlock.

Appendix Q–II–C–2–h. Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated every 30 days with an indicator organism.

Appendix Q–II–C–2–i. An exhaust air ventilation system shall be provided. This system shall create directional airflow that draws air into the animal room through the entry area. The building exhaust, or the exhaust from primary containment units, may be used for this purpose if the exhaust air is discharged to the outside and shall be dispersed away from occupied areas and air intakes. Personnel shall verify that the direction of the airflow (into the animal room) is proper.

Appendix Q–II–C–2–j. If the agent is transmitted by aerosol, then the exhaust air shall pass through a high efficiency particulate air/HEPA filter.

Appendix Q–II–C–2–k. Vacuum lines shall be protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.

Appendix Q–II–C–2–l. In lieu of open housing in the special animal room, animals held in a BL3–N area may be housed in partial-containment caging systems (e.g., Horsfall units or gnotobiotic systems, or other special containment primary barriers). Prudent judgment must be exercised to implement this ventilation system (e.g., animal species) and its discharge location.

Appendix Q–II–C–2–m. Each animal area shall contain a foot, elbow, or automatically operated sink for hand washing. The sink shall be located near the exit door.

Appendix Q–II–C–2–n. Restraining devices for animals may be required to avoid damage to the integrity of the animal containment facility.

Appendix Q–II–D. Biosafety Level 4—Animals (BL4–N) (See Appendix Q–III–C)

Appendix Q–II–D–1. Standard Practices (BL4–N)

Appendix Q–II–D–1–a. Animal Facility Access (BL4–N)

Appendix Q–II–D–1–a–(1). Individuals under 16 years of age shall not be permitted to enter the animal area.

Appendix Q–II–D–1–a–(2). The containment area shall be locked.

Appendix Q–II–D–1–a–(3). The containment area shall be patrolled or monitored at frequent intervals.

Appendix Q–II–D–1–a–(4). The containment building shall be controlled and have a locking access.

Appendix Q–II–D–1–a–(5). The Animal Facility Director shall establish policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) may enter the laboratory or animal room.

Appendix Q–II–D–1–a–(6). Individuals shall enter and exit the animal facility only through the clothing change and shower rooms.

Appendix Q–II–D–1–a–(7). Personnel shall use the airlocks to enter or exit the laboratory only in an emergency.

Appendix Q–II–D–1–a–(8). Animal room doors, gates, and other closures shall be kept closed when experiments are in progress.

Appendix Q–II–D–1–b. Decontamination and Inactivation (BL4–N)

Appendix Q–II–D–1–b–(1). All contaminated liquid or solid wastes shall be decontaminated before disposal.

Appendix Q–II–D–1–b–(2). The work surfaces and containment equipment shall be decontaminated when work with organisms containing recombinant DNA molecules is finished. Where feasible, plastic-backed paper toweling shall be used on nonporous work surfaces to facilitate clean-up.

Appendix Q–II–D–1–b–(3). All wastes from animal rooms and laboratories shall be appropriately decontaminated before disposal in an approved manner.

Appendix Q–II–D–1–b–(4). No materials, except for biological materials that are to remain in a viable or intact state, shall be removed from the maximum containment laboratory unless they have been autoclaved or decontaminated.

Equipment or material that might be damaged by high temperatures or steam shall be decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

Appendix Q-II-D–1-b-(5). When ventilated suits are required, the animal personnel shower entrance/exit area shall be equipped with a chemical disinfectant shower to decontaminate the surface of the suit before exiting the area. A neutralization or water dilution device shall be integral with the chemical disinfectant discharge piping before entering the heat sterilization system. Entry to this area shall be through an airlock fitted with airtight doors.

Appendix Q-II-D–1-b-(6). Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q-II-D–1-b-(7). Supplies and materials needed in the animal facility shall be brought in by way of the double-door autoclave, fumigation chamber, or airlock that shall be appropriately decontaminated between each use.

Appendix Q-II-D–1-b-(8). An autoclave, incinerator, or other effective means to decontaminate animals and wastes shall be available, preferably within the containment area. If feasible, a double-door autoclave is preferred and should be positioned to allow removal of material from the containment area.

Appendix Q-II-D–1-b-(9). Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. Liquid wastes from shower rooms and toilets shall be decontaminated with chemical disinfectants or heat by methods demonstrated to be effective. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated every 30 days with an indicator organism. Liquid wastes from the shower shall be chemically decontaminated using an Environmental Protection Agency-approved germicide. The efficacy of the chemical treatment process shall be validated with an indicator organism. Chemical disinfectants shall be neutralized or diluted before release into general effluent waste systems.

Appendix Q-II-D–1-c. Signs (BL4–N)

Appendix Q-II-D–1-c-(1). When the animal research requires special provisions for entry (e.g., vaccination), a warning sign incorporating the universal biosafety symbol shall be posted on all access doors to the animal work area. The sign shall indicate: (i) The agent, (ii) the animal species, (iii) the name and telephone number of the Animal Facility Director, or other responsible individual, and (iv) any special requirements for entering the laboratory.

Appendix Q-II-D–1-d. Protective Clothing (BL4–N)

Appendix Q-II-D–1-d-(1). Individuals shall enter and exit the animal facility only through the clothing change and shower rooms. Street clothing shall be removed and kept in the outer clothing change room. Complete laboratory clothing (may be disposable), including undergarments, pants, shirts, jump suits, and shoes shall be provided for all personnel entering the animal facility. When exiting the BL4–N area and before proceeding into the shower area, personnel shall remove their laboratory clothing in the inner change room. All laboratory clothing shall be autoclaved before laundering. Personnel shall shower each time they exit the animal facility.

Appendix Q-II-D–1-d-(2). A ventilated head-hood or a one-piece positive pressure suit, which is ventilated by a life-support system, shall be worn by all personnel entering rooms that contain experimental animals when appropriate. When ventilated suits are required, the animal personnel shower entrance/exit area shall be equipped with a chemical disinfectant shower to decontaminate the surface of the suit before exiting the area. A neutralization or water dilution device shall be integral with the chemical disinfectant discharge piping before entering the heat sterilization system. Entry to this area shall be through an airlock fitted with airtight doors.

Appendix Q-II-D–1-d-(3). Appropriate respiratory protection shall be worn in rooms containing experimental animals.

Appendix Q-II-D–1-e. Records (BL4–N)

Appendix Q-II-D–1-e-(1). Documents regarding experimental animal use and disposal shall be maintained in a permanent record book.

Appendix Q-II-D–1-e-(2). A system shall be established for: (i) Reporting laboratory accidents and exposures that are a result of overt exposures to organisms containing recombinant DNA, (ii) employee absenteeism, and (iii) medical surveillance of potential laboratory-associated illnesses. Permanent records shall be prepared and maintained. Any incident involving spills and accidents that results in environmental release or exposures of animals or laboratory workers to organisms containing recombinant DNA molecules shall be reported immediately to the Biological Safety Officer, Animal Facility Director, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities (if applicable). Reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496–9838. Medical evaluation, surveillance, and treatment shall be

provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.

Appendix Q-II-D–1-e-(3). When appropriate and giving consideration to the agents handled, baseline serum samples shall be collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agents handled or the function of the facility.

Appendix Q-II-D–1-e-(4). A permanent record book indicating the date and time of each entry and exit shall be signed by all personnel.

Appendix Q-II-D–1-f. Transfer of Materials (BL4–N)

Appendix Q-II-D–1-f-(1). No materials, except for biological materials that are to remain in a viable or intact state, shall be removed from the maximum containment laboratory unless they have been autoclaved or decontaminated. Equipment or material that might be damaged by high temperatures or steam shall be decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

Appendix Q-II-D–1-f-(2). Biological materials removed from the animal maximum containment laboratory in a viable or intact state shall be transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container that shall be removed from the animal facility through a disinfectant dunk tank, fumigation chamber, or an airlock designed for this purpose. Advance approval for transfer of material shall be obtained from the Animal Facility Director. Such packages containing viable agents can only be opened in another BL4–N animal facility if the agent is biologically inactivated or incapable of reproduction. Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval shall be required to transfer agents or tissue/organ specimens from a BL4–N animal facility to one with a lower containment classification.

Appendix Q-II-D–1-f-(3). Supplies and materials needed in the animal facility shall be brought in by way of the double-door autoclave, fumigation chamber, or airlock that shall be appropriately decontaminated between each use. After securing the outer doors, personnel within the animal facility retrieve the materials by opening the interior doors of the autoclave, fumigation chamber, or airlock. These doors shall be secured after materials are brought into the animal facility.

Appendix Q-II-D–1-g. Other (BL4–N)

Appendix Q-II-D–1-g-(1). All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

Appendix Q-II-D–1-g-(2). Eating, drinking, smoking, and applying cosmetics shall not be permitted in the work area.

Appendix Q-II-D–1-g-(3). Individuals who handle materials and animals containing recombinant DNA molecules shall be required to wash their hands before exiting the containment area.

Appendix Q-II-D–1-g-(4). Experiments involving other organisms that require containment levels lower than BL4–N may be conducted in the same area concurrently with experiments requiring BL4–N containment provided that they are conducted in accordance with BL4–N practices.

Appendix Q-II-D–1-g-(5). Animal holding areas shall be cleaned at least once a day and decontaminated immediately following any spill of viable materials.

Appendix Q-II-D–1-g-(6). All procedures shall be performed carefully to minimize the creation of aerosols.

Appendix Q-II-D–1-g-(7). A double barrier shall be provided to separate male and female animals. Animal isolation barriers shall be sturdy and accessible for cleaning. Reproductive incapacitation may be used.

Appendix Q-II-D–1-g-(8). The containment area shall be in accordance with state and Federal laws and animal care requirements.

Appendix Q-II-D–1-g-(9). The life support system for the ventilated suit or head hood is equipped with alarms and emergency back-up air tanks. The exhaust air from the suit area shall be filtered by two sets of high efficiency particulate air/HEPA filters installed in series or incinerated. A duplicate filtration unit, exhaust fan, and an automatically starting emergency power source shall be provided. The air pressure within the suit shall be greater than that of any adjacent area. Emergency lighting and communication systems shall be provided. A double-door autoclave shall be provided for decontamination of waste materials to be removed from the suit area.

Appendix Q-II-D–1-g-(10). Needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) shall be used for the injection or aspiration of fluids containing organisms that contain recombinant DNA. Extreme caution shall be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Following use, needles shall not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe. The needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q-II-D–1-g-(11). An essential adjunct to the reporting-surveillance system is the availability of a facility for quarantine, isolation, and medical care of personnel with potential or known laboratory-associated illnesses.

Appendix Q-II-D–1-g-(12). A biosafety manual shall be prepared or adopted. Personnel shall be advised of special hazards and required to read and follow instructions on practices and procedures.

Appendix Q-II-D–1-g-(13). Vacuum lines shall be protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.

Appendix Q-II-D–2. Animal Facilities (BL4–N)

Appendix Q-II-D–2-a. Animals shall be contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and avoid arthropod access.

Appendix Q-II-D–2-b. The interior walls, floors, and ceilings shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat, to facilitate cleaning. Penetrations in these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

Appendix Q-II-D–2-c. Windows in the animal facility shall be closed, sealed, and breakage resistant (e.g., double-pane tempered glass or equivalent).

Appendix Q-II-D–2-d. An autoclave, incinerator, or other effective means to decontaminate animals and wastes shall be available, preferably within the containment area. If feasible, a double-door autoclave is preferred and should be positioned to allow removal of material from the containment area.

Appendix Q-II-D–2-e. Access doors to the containment area shall be self-closing.

Appendix Q-II-D–2-f. All perimeter joints and openings shall be sealed to form an arthropod-proof structure.

Appendix Q-II-D–2-g. The BL4–N laboratory provides a double barrier to prevent the release of recombinant DNA containing microorganisms into the environment. Design of the animal facility shall be such that if the barrier of the inner facility is breached, the outer barrier will prevent release into the environment. The animal area shall be separated from all other areas. Passage through two sets of doors shall be the basic requirement for entry into the animal area from access corridors or other contiguous areas. Physical separation of the animal containment area from access corridors or other laboratories or activities shall be provided by a double-door clothes change room equipped with integral showers and airlock.

Appendix Q-II-D–2-h. A necropsy room shall be provided within the BL4–N containment area.

Appendix Q-II-D–2-i. Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. Liquid wastes from shower rooms and toilets shall be decontaminated with chemical disinfectants or heat by methods demonstrated to be effective. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated every 30 days with an indicator organism. Liquid wastes from the shower shall be chemically decontaminated using an Environmental Protection Agency-approved germicide.

The efficacy of the chemical treatment process shall be validated with an indicator organism. Chemical disinfectants shall be neutralized or diluted before release into general effluent waste systems.

Appendix Q-II-D–2-j. A ducted exhaust air ventilation system shall be provided that creates directional airflow that draws air into the laboratory through the entry area. The exhaust air, which is not recirculated to any other area of the building, shall be discharged to the outside and dispersed away from the occupied areas and air intakes. Personnel shall verify that the direction of the airflow (into the animal room) is proper.

Appendix Q-II-D–2-k. Exhaust air from BL4–N containment area shall be double high efficiency particulate air/HEPA filtered or treated by passing through a certified HEPA filter and an air incinerator before release to the atmosphere. Double HEPA filters shall be required for the supply air system in a BL4–N containment area.

Appendix Q-II-D–2-l. All high efficiency particulate air/HEPA filters' frames and housings shall be certified to have no detectable smoke [dioctylphthalate] leaks when the exit face (direction of flow) of the filter is scanned above 0.01 percent when measured by a linear or logarithmic photometer. The instrument must demonstrate a threshold sensitivity of at least 1×10^{-3} micrograms per liter for 0.3 micrometer diameter dioctylphthalate particles and a challenge concentration of 80–120 micrograms per liter. The air sampling rate should be at least 1 cfm (28.3 liters per minute).

Appendix Q-II-D–2-m. If an air incinerator is used in lieu of the second high efficiency particulate air/HEPA filter, it shall be biologically challenged to prove all viable test agents are sterilized. The biological challenge must be minimally 1×10^{8} organisms per cubic foot of airflow through the incinerator. It is universally accepted if bacterial spores are used to challenge and verify that the equipment is capable of killing spores, then assurance is provided that all other known agents are inactivated by the parameters established to operate the equipment. Test spores meeting this criterion are *Bacillus subtilis* var. *niger* or *Bacillus stearothermophilis*. The operating temperature of the incinerator shall be continuously monitored and recorded during use.

Appendix Q-II-D–2-n. All equipment and floor drains shall be equipped with deep traps (minimally 5 inches). Floor drains shall be fitted with isolation plugs or fitted with automatic water fill devices.

Appendix Q-II-D–2-o. Each animal area shall contain a foot, elbow, or automatically operated sink for hand washing. The sink shall be located near the exit door.

Appendix Q–II–D–2–p. Restraining devices for animals may be required to avoid damage to the integrity of the containment animal facility.

Appendix Q–II–D–2–q. The supply water distribution system shall be fitted with a back-flow preventer or break tank.

Appendix Q–II–D–2–r. All utilities, liquid and gas services, shall be protected with devices that avoid back-flow.

Appendix Q–II–D–2–s. Sewer and other atmospheric ventilation lines shall be equipped minimally with a single high efficiency particulate/HEPA filter. Condensate drains from these type housings shall be appropriately connected to a contaminated or sanitary drain system. The drain position in the housing dictates the appropriate system to be used.

Appendix Q–III. Footnotes and References for Appendix Q

Appendix Q–III–A. If recombinant DNA is derived from a Class 2 organism requiring BL2 containment, personnel shall be required to have specific training in handling pathogenic agents and directed by knowledgeable scientists.

Appendix Q–III–B. Personnel who handle pathogenic and potentially lethal agents shall be required to have specific training and be supervised by knowledgeable scientists who are experienced in working with these agents. BL3–N containment also minimizes escape of recombinant DNA-containing organisms from exhaust air or waste material from the containment area.

Appendix Q–III–C. Classes 4 and 5 microorganisms pose a high level of individual risk for acquiring life-threatening diseases to personnel and/or animals. To import Class 5 agents, special approval must be obtained from U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Import-Export Products, Room 756, Federal Building, 6505 Belcrest Road, Hyattsville, Maryland 20782.

Laboratory staff shall be required to have specific and thorough training in handling extremely hazardous infectious agents, primary and secondary containment, standard and special practices, and laboratory design characteristics. The laboratory staff shall be supervised by knowledgeable scientists who are trained and experienced in working with these agents and in the special containment facilities.

Within work areas of the animal facility, all activities shall be confined to the specially equipped animal rooms or support areas. The maximum animal containment area and support areas shall have special engineering and design features to prevent the dissemination of microorganisms into the environment via exhaust air or waste disposal.

Appendix Q–III–D. Other research with non-laboratory animals, which may not appropriately be conducted under conditions described in Appendix Q, may be conducted safely

by applying practices routinely used for controlled culture of these biota. In aquatic systems, for example, BL1 equivalent conditions could be met by utilizing growth tanks that provide adequate physical means to avoid the escape of the aquatic species, its gametes, and introduced exogenous genetic material. A mechanism shall be provided to ensure that neither the organisms nor their gametes can escape into the supply or discharge system of the rearing container (e.g., tank, aquarium, etc.) Acceptable barriers include appropriate filtration, irradiation, heat treatment, chemical treatment, etc. Moreover, the top of the rearing container shall be covered to avoid escape of the organism and its gametes. In the event of tank rupture, leakage, or overflow, the construction of the room containing these tanks should prevent the organisms and gametes from entering the building's drains before the organism and its gametes have been inactivated.

Other types of non-laboratory animals (e.g., nematodes, arthropods, and certain forms of smaller animals) may be accommodated by using the appropriate BL1 through BL4 or BL1–P through BL4–P containment practices and procedures as specified in Appendices G and P.

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.

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Harold Varmus,

Director, National Institutes of Health.

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