INC., 31 Frederick Place, Old Bridge, NJ 08857. Transferor: Bivins Freight Service, Inc., South 15th Street, Millville, NJ 08332. Representative: Piken & Piken, Attorneys-at-Law, One Lefrak City Plaza, Flushing, NY 11368. Authority sought for purchase by transferee of the operating rights of transferor, as set forth in Certificate MC 73618, issued March 20, 1963, as follows: *General commodities* (with the usual exceptions), over regular routes, between Millville, NJ and Philadelphia, PA, and other specified commodities excepted. *Building material, and brick, feed, grain and meat scraps, seed, fertilizer, fertilizer material, airplane engines, airplane engine parts, and airplane accessories, boat building materials, equipment, and supplies,* over irregular routes, generally between Millville and other specified cities in NJ and specified cities in NY, MD, PA, DE, and DC. Transferee presently holds no authority from this Commission. Application has not been filed for temporary authority under section 210a(b).

MC-FC-77716, filed June 21, 1978. Transferee: Graham Bell, d.b.a. B & W Trucking, P.O. Box 281, 462 Essex Avenue, Gloucester, MA 01903. Transferor: Roger D. Peterson, d.b.a. Peterson Motor Transportation, 107 Portland Street, Rochester, NH. Representative: George C. O'Brien, Attorney-at-Law, 12 Vernon Street, Norwood, MA 02062. Authority sought for purchase by transferee of a portion of the operating rights set forth in Certificate MC 7953, issued November 18, 1975, as follows: *General commodities* (with usual exceptions), over regular routes, between Boston, and Haverhill, MA, serving all intermediate and specified and off-route points; *radio tubes* and *supplies* and *containers* for such commodities during the season extending from the 15th of May to the 15th of September, between Salem, MA and Corning, NY serving no intermediate points; over irregular routes, *general commodities* (with usual exceptions), between Boston, Quincy, Hingham, Milton, and Weymouth, MA; *supplies, materials, equipment,* and *machinery* used in the manufacture of lumber and lumber products, between Providence, RI and Boston, MA on the one hand, and, on the other, Rochester, NH; *Such merchandise* as is dealt in by wholesale, retail, and chain grocery and food business houses, and in connection therewith, equipment, materials, and supplies used in the conduct of such business, between points in Strafford, Rockingham, and Carroll Counties, NH, on the one hand, and, on the other, points in Androscoggin, Cumberland, Oxford, and York Counties, ME; from points in Dover, Portsmouth, and Rochester, NH to points in a described portion of NH; from Rochester, NH to Wallum Lake, RI; groceries and grocery supplies, fruit, and vegetables, from Boston MA to Rochester, NH and South Berwick, ME; *building materials,* from Walpole and Boston, MA and points within 5 miles of Boston, to Rochester; *box shooks,* from Rollinsord, NH to Gloucester, MA and *rejected shipments of box shooks,* from Gloucester, MA to Rollinsford, NH; *wooden box shooks* and *sawdust,* from Rochester, NH to Willimantic, CT; and *metal screws* used in the manufacture of wooden boxes, from Willimantic, CT to Rochester, NH. Transferee holds no Commission authority and does not seek section 210a(b) temporary authority.

H. G. HOMME, Jr.,
Acting Secretary.

[FR Doc. 78-20946 Filed 7-27-78; 8:45 am]

[7035-01]

[Notice No. 92]

MOTOR CARRIER TRANSFER PROCEEDINGS

JULY 28, 1978.

Application filed for temporary authority under section 210a(b) in connection with transfer application under section 212(b) and transfer rules, 49 C.F.R. Part 1132:

MC-FC 77651. By application filed July 5, 1978, OIL COUNTRY HAULERS, INC., 15714 Old Beaumont Hwy (U.S. 90), Houston, TX 77049, seeks temporary authority to transfer the operating rights of SHELDON TRUCKING CO., 15714 Old Beaumont Hwy (U.S. 90), Houston, TX 77049, under section 210a(b). The transfer to OIL COUNTRY HAULERS, INC., of the operating rights of SHELDON TRUCKING CO., is presently pending.

MC-FC 77756. By application filed July 12, 1978, C & H BUS LINES, INC., Route 1, Harrison, GA 31035, seeks temporary authority to transfer the operating rights of NATIONAL BUS SERVICE, INC., 746 Wheaton Street, Savannah, GA 31401, under section 210a(b). The transfer to C & H BUS LINES, INC., of the operating rights of NATIONAL BUS SERVICE, INC., is presently pending.

MC-FC 77757. By application filed July 18, 1978, McNULTY TANK LINES DIVISION, McNULTY INDUSTRIES, INC., d.b.a. McNULTY TANK LINES, U.S. Hwy 130, Bridgeport, NJ 08014, seeks temporary authority to transfer the operating rights of SKYLINE TRANSPORT, INC., 1910 Russell Street, Baltimore, MD 21230, under section 210a(b). The transfer to McNULTY TANK LINES DIVISION, McNULTY INDUSTRIES, INC., d.b.a. McNULTY TANK LINES, of the operating rights of SKYLINE TRANSPORT, INC., is presently pending.

By the Commission.

H. G. HOMME, Jr.,
Acting Secretary.

[FR Doc. 78-20947 Filed 7-27-78; 8:45 am]

sunshine act meetings

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).

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[6351-01]

1

COMMODITY FUTURES TRADING COMMISSION.

TIME AND DATE: 10 a.m., July 28, 1978.

PLACE: 2033 K Street NW., Washington, D.C., 5th floor hearing room.

STATUS: Closed.

MATTERS TO BE CONSIDERED: Discussion of an *amicus curiae* memorandum.

CONTACT PERSON FOR MORE INFORMATION:

Jane Stuckey, 254-6314.

[S-1545-78 Filed 7-26-78; 11:44 am]

[6351-01]

2

COMMODITY FUTURES TRADING COMMISSION.

TIME AND DATE: 11 a.m., August 4, 1978.

PLACE: Eighth floor conference room, 2033 K Street NW., Washington, D.C.

STATUS: Closed.

MATTERS TO BE CONSIDERED: Market surveillance.

CONTACT PERSON FOR MORE INFORMATION:

Jane Stuckey, 254-6314.

[S-1546-78 Filed 7-26-78; 11:44 am]

[6714-01]

3

FEDERAL DEPOSIT INSURANCE CORPORATION.

TIME AND DATE: 10 a.m., August 2, 1978.

PLACE: Room 6135, FDIC Building, 550 17th Street NW., Washington, D.C.

STATUS: Closed.

MATTERS TO BE CONSIDERED:

Applications for Federal deposit insurance:

NBD-Portage Bank, a proposed new bank to be located at 6400 South Westnedge Avenue, Portage, Mich., for Federal deposit insurance.

Banco Union de Puerto Rico, a proposed new bank to be located on Ignacio Arzuaga Street, corner of Bernardo Garcia Street, Carolina, Puerto Rico, for Federal deposit insurance.

Application for Federal deposit insurance and for consent to establish a branch (drive-in facility):

Burbank Citizens Bank, a proposed new bank to be located at 333 North Glenoaks Boulevard, Burbank, Calif., for Federal deposit insurance and for consent to establish a branch (drive-in facility) at 372 East Olive Avenue, Burbank, Calif.

Applications for Federal deposit insurance and for consent to exertise limited trust powers:

First Bank & Trust of Carter Lake, a proposed new bank to be located at 1230 Locust Street, Carter Lake, Iowa, for Federal deposit insurance and for consent to exercise limited trust powers.

Applications for consent to establish branches:

Dixie County State Bank, Crose City, Fla., for consent to establish a branch at the northeast corner of State Road 358, south end of the river bridge approach, Unincorporated Jena Area of Dixie County, Fla.

Umatilla State Bank, Umatilla Fla., for consent to establish a branch on Butler Street (State Road 40) near its intersection with Alco Street, unincorporated Lake County (P.O. Astor), Fla.

The Citizens Bank of Perry, Perry, Fla., for consent to establish a branch in the southwest quadrant of the intersection of Ninth Street East and First Avenue, unincorporated area of Steinhatchee, Taylor County, Fla.

United Mutal Savings Bank, New York, N.Y., for consent to establish a branch at 556 Main Street, Islip (unincorporated area), town of Islip, N.Y.

The Western Saving Fund Society of Philadelphia, Haverford, Pa., for consent to establish a branch at 2601-05 South Broad Street, Philadelphia, Pa.

Application for consent to establish a branch-detached facility:

The Kiowa State Bank, Kiowa, Colo., for consent to establish a branch-detached facility on Highway 86, Elizabeth, Colo.

Applications for consent to merge and to establish branches:

Albany Savings Bank, Albany, N.Y., an insured mutal savings bank, for consent to merge under its charter and title with Onedia Federal Savings & Loan Association, Oneida, N.Y., upon the latter's conversion to a State charter, and for consent to establish the sole office of the latter institution as a branch of Albany Savings Bank.

Brookville Bank & Trust Co., Brookville, Pa., an insured State nonmember bank, for consent to merge under its charter, and with the title of "Unibank," with Brockway Citizens Bank, Brockway, Pa., also an insured State nonmember bank, and for consent to establish the one existing office of Brockway Citizens Bank as a branch of the resultant bank.

Farmers Bank & Trust Co., of Hanover, Hanover, Pa., an insured State nonmember bank, for consent to merge under its charter and title with Abbottstown State Bank, Abbottstown, Pa., also an insured State nonmember bank, and for consent to establish the sole office of Abbottstown State Bank as a branch of the resultant bank.

Application for consent to consolidate, establish branches, redesignate the main office location, and exercise trust powers:

Southwest Mississippi Bank, Magnolia, Miss., an insured State nonmember bank, for consent to consolidate under its charter, and with the title of "First Bank of Southwest Mississippi," with Bank of McComb, McComb, Miss., also an insured State nonmember bank; for consent to establish Bank of McComb's four offices as branches of the resultant bank; for consent to redesignate its main office location to the present site of the main office of Bank of McComb; and for consent to exercise trust powers.

Recommendations regarding liquidation of a bank's assets acquired by the Corporation in its capacity as receiver, liquidator, or liquidating agent of those assets:

Case No. 43,549-L—Bank of Picayune, Picayune, Miss.

Case No. 43,580-L—Fanklin National Bank, New York, N.Y.

Case No. 43,584-NR—United States National Bank, San Diego, Calif.

Case No. 43,588-L—First State Bank Of Northern California, San Leandro, Calif.

Case No. 43,590-L—American City Bank & Trust Co., National Association, Milwaukee, Wis.

Case No. 43,591-L—The Drovers' National Bank of Chicago, Chicago, Ill.

Case No. 43,592-L—The Drovers' National Bank of Chicago, Chicago, Ill.
Case No. 43,596-SR—Ill.The Peoples Bank of the Virgin Islands, Charlotte Amalie, Virgin Islands.
Case No. 43,595-L—The Drovers' National Bank of Chicago, Chicago, Ill.

Recommendations with respect to the initiation or termination of cease-and-desist proceedings, termination-of-insurance proceedings, or suspension or removal proceedings against certain insured banks or officers or directors thereof:
Names of persons and names and locations of banks authorized to be exempt from disclosure pursuant to the provisions of subsections (c)(6), (c)(8), and (c)(9)(A)(ii) of the "Government in the Sunshine Act" (5 U.S.C. 552b (c)(6), (c)(8), and (e)(9)(A)(ii)).

Personnel actions regarding appointments, promotions, administrative pay increases, reassignments, retirements, separations, removals, etc.
Names of employees authorized to be exempt from disclosure pursuant to the provisions of subsections (c)(2) and (c)(6) of the "Government in the Sunshine Act" (5 U.S.C. 552b (c)(2) and (c)(6)).

Grievance officer's findings and recommendations in connection with the formal grievance of a corporation employee:
Name of employee authorized to be exempt from disclosure pursuant to the provisions of subsection (c)(6) of the "Government in the Sunshine Act" (5 U.S.C. 552b(c)(6)).

CONTACT PERSON FOR MORE INFORMATION:

Alan R. Miller, Executive Secretary, 202-389-4446.

[S-15550-78 Filed 7-26-78; 3:54 pm]

[6714-01]

4

FEDERAL DEPOSIT INSURANCE CORPORATION.

TIME AND DATE: 10:30 a.m., August 2, 1978.

PLACE: Board room, sixth floor, FDIC Building, 550 17th Street NW., Washington, D.C.

STATUS: Open.

MATTERS TO BE CONSIDERED:

Disposition of minutes of previous meetings.
Application for Federal deposit insurance:
Hickory Point Bank, a proposed new bank to be located in the Hickory Point Mall at the intersection of U.S. Highway 51 and Interstate Highway 72, Forsyth, Ill., for Federal deposit insurance.

Request by the Comptroller of the Currency for a report on the competitive factors involved in the proposed merger of Adams County National Bank, Cumberland Township (P.O. Gettysburg), Pa., and The National Bank of Arendtsville, Arendtsville, Pa.

Recommendations with respect to payment for legal services rendered and expenses incurred in connection with receivership and liquidation activities:
Schall, Boudreau & Gore, San Diego, Calif., in connection with the receivership of United States National Bank, San Diego, Calif.
Trager & Trager, Fairfield, Conn., in connection with the liquidation of the Monroe Bank & Trust Co., Monroe, Conn.
Sullivan & Worcester, Boston, Mass., in connection with the receivership of Surety Bank & Trust Co., Wakefield, Mass.
Kaye, Scholer, Pierman, Hays & Handler, New York., N.Y., in connection with the liquidation of Franklin National Bank, New York, N.Y.
O'Neill & Borges, Raco Rey, Puerto Rico, in connection with the liquidation of Banco Credito y Ahorro Ponceno, Ponce, P.R.

Memorandum and resolution proposing certain delegations of authority from the Board of Directors to the Committee on Liquidations, Loans and Purchases of Assets (Case No. 43,386).

Memorandum and resolution proposing the delegation to the General Counsel and the Director of the Division of Liquidation, or their designees, of authority to initiate litigation to which the Corporation will be a party either in its corporate capacity or as receiver of a closed bank.

Memorandum proposing the procurement of new computer equipment.

Memorandums and resolutions proposing that the liquidators of Banco Economias, San German, Puerto Rico, and Banco Credito y Ahorro Ponceno, Ponce, Puerto Rico, be authorized to convey real property.

Reports of Committees and Officers

Minutes of the actions approved by the Committee on Liquidations, Loans, and Purchases of Assets pursuant to authority delegated by the Board of Directors.
Report of the Executive Secretary regarding his transmittal of "no significant effect" competitive factor reports.
Reports of the directory of the Division of Bank Supervision with respect to applications or requests approved by him and the various Regional Directors pursuant to authority delegated by the Board of Directors.
Final report of the Office of the Controller on the termination of the liquidation of First Citizens Bank & Trust Co. of Utica, Utica, N.Y.
Reports of security transactions authorized by the Chairman.

CONTACT PERSON FOR MORE INFORMATION:

Alan R. Miller, Executive Secretary, 202-389-4446.

[S-1551-78 Filed 7-26-78; 3:54 pm]

[6715-01]

5

FEDERAL ELECTION COMMISSION.

DATE AND TIME: Wednesday, August 2, 1978, at 10 a.m.

PLACE: 1325 K Street NW., Washington, D.C.

STATUS: This meeting will be closed to the public.

MATTERS TO BE CONSIDERED: Audits, compliance, and personnel.

* * * * *

DATE AND TIME: Thursday, August 3, 1978, at 10 a.m.

MATTERS TO BE CONSIDERED:

Portions open to the public:

Setting of Future Meeting Dates
Correction and Approval of Minutes
Advisory Opinion 1978-42
Advisory Opinion 1978-48
Response to Advisory Opinion Request From National Treasury Employees Union
Policy Regarding Transfers to Registered Entities From Unregistered Organizations
Quarterly Management Report
Pending Legislation
Pending Litigation
Appropriations and Budget
Liaison With Other Federal Agencies
Classification Actions
Routine Administrative Matters

Portions closed to the public (executive session):

Any Matters Not Concluded on August 2, 1978.

PERSON TO CONTACT FOR INFORMATION:

Mr. David Fiske, Press Officer, telephone 202-523-4065.

MARJORIE W. EMMONS,
Secretary to the Commission.

[S-1549-78 Filed 7-26-78; 3:54 pm]

[6740-02]

6

FEDERAL ENERGY REGULATORY COMMISSION.

"FEDERAL REGISTER" CITATION OF PREVIOUS ANNOUNCEMENT: (Published July 24, 1978; 43 FR 32025).

PREVIOUSLY ANNOUNCED TIME AND DATE OF MEETING: 10 a.m., July 26, 1978.

CHANGE IN THE MEETING: The following items have been added:

Item No., Docket No., and Company

M-4—Memorandum of Understanding between the Secretary of Energy and the Federal Energy Regulatory Commission concerning procedures for review of proposed rules, regulations, and statements of policy.
M-5—RM78-40, Natural Gas Companies' Annual Report of Proved Domestic Gas Reserves: FPC No. 40—extension of 1977 filing deadlines.

C1-4—CI75-45, et al., Tenneco Oil Co., et al.

KENNETH F. PLUMB,
Secretary.

[S-1547-78 Filed 7-26-78; 11:44 am]

[6720-01]

7

FEDERAL HOME LOAN BANK BOARD.

TIME AND DATE: 9:30 a.m., August 2, 1978.

PLACE: 1700 G Street NW., Sixth Floor, Washington, D.C.

STATUS: Open meeting.

CONTACT PERSON FOR MORE INFORMATION:

Franklin O. Bolling, 202-377-6677.

MATTERS TO BE CONSIDERED:

Consideration of Final Regulations Re: FTC Pre-Merger Notification Requirements.
Consideration of Amendments Relating to Servicing of Loans.
Consideration of Waiver of Condition Re: Branch Office Opening—First Federal Savings & Loan Association of Pineville, Pineville, Ky.
Consideration of Proposed Acquisition of Western Savings Association, Pratt, Kans., and Larned Savings & Loan Association, Larned, Kans., by Western Financial Corp., Pratt, Kans.
Applications for Bank Membership and Insurance of Accounts—Evergreen Savings & Loan Association, Redwood City, Calif.
Branch Office Application—First Federal Savings & Loan Association of Brunswick, Brunswick, Ga.

No. 169, July 26, 1978.

[S-1553-78 Filed 7-26-78; 3:54 pm]

[6730-01]

8

FEDERAL MARITIME COMMISSION.

"FEDERAL REGISTER" CITATION OF PREVIOUS ANNOUNCEMENT: July 21, 1978, 43 FR 31503.

PREVIOUSLY ANNOUNCED TIME AND DATE OF THE MEETING: 10 a.m., July 26, 1978.

CHANGES IN THE MEETING: Addition of the following item to the closed session:

3. Practices of Zim-American Israeli Shipping Co., Inc.

[S-1543-78 Filed 7-26-78; 11:50 am]

[6730-01]

9

FEDERAL MARITIME COMMISSION.

TIME AND DATE: August 2, 1978—10 a.m.

PLACE: Room 12126—1100 L Street NW., Washington, D.C. 20573.

STATUS: Open.

MATTERS TO BE CONSIDERED:

1. Agreement No. 8660-8: Modification of the Latin America/Pacific Coast Steamship Conference to include intermodal service.
2. Agreement No. 9510-4: Modification of the Household Goods Forwarders Association of America Rate Agreement to permit independent action of the members.
2. Agreement No. 7680-D.R.: Petition of American West African Freight Conference for unlimited extension dual rate contract.
4. Agreement No. 9973-4: Modification of Johnson ScanStar Combined Service Agreement to permit service to the Pacific Coast via minibridge.
5. Petition for Declaratory Order—Dr. Jenaro Collazo, Secretary of Social Services of Puerto Rico (Petitioner).
6. Petition for Reconsideration of Agreements Nos. 2846-28 and 5660-21.
7. Docket No. 76-60: Petition for Declaratory Order of Seatrain International, S.A.—Consideration of record.
8. Docket No. 70-50: Marine Terminal Practices of the Port of Seattle—Possible violation of section 17, Shipping Act, 1916—Consideration of initial decision.

CONTACT PERSON FOR MORE INFORMATION:

Francis C. Hurney, Secretary, 202-523-5725.

[S-1544-78 Filed 7-26-78; 11:44 am]

[6210-01]

10

BOARD OF GOVERNORS OF THE FEDERAL RESERVE SYSTEM.

TIME AND DATE: 10 a.m., Wednesday, August 2, 1978.

PLACE: 20th Street and Constitution Avenue NW., Washington, D.C. 20551.

STATUS: Open.

MATTERS TO BE CONSIDERED: Summary Agenda: Because of their routine nature, no substantive discussion of the following items is anticipated. These matters will be resolved with a single vote unless a member of the Board requests that an item be moved to the discussion agenda:

1. Request for an exemption from the Federal Home Mortgage Disclosure Act and Regulation C for Connecticut chartered depository institutions that are subject to the Connecticut Home Mortgage Disclosure Act and the implementing regulations of the Connecticut Banking Department.
2. Proposed extension and revision of the Commercial Bank Report of Consumer Credit (FR 571).

Discussion Agenda:

1. Proposed statement to be presented to the Senate Committee on Banking, Housing, and Urban Affairs regarding S. 2011, the Regulatory Reduction and Congressional Control Act.
2. Any agenda items carried forward from a previously announced meeting.

CONTACT PERSON FOR MORE INFORMATION:

Mr. Joseph R. Coyne, Assistant to the Board, 202-452-3204.

Dated: July 25, 1978.

GRIFFITH L. GARWOOD,
Deputy Secretary of the Board.

[S-1552-78 Filed 7-26-78; 3:54 pm]

[7020-02]

11

[USITC SE-78-36A]

UNITED STATES INTERNATIONAL TRADE COMMISSION.

"FEDERAL REGISTER" CITATION OF PREVIOUS ANNOUNCEMENT: 43 FR 32220, July 25, 1978.

PREVIOUSLY ANNOUNCED TIME AND DATE OF THE MEETING: 10 a.m., Thursday, August 3, 1978.

CHANGES IN THE MEETING: Enlargement of the scope of item No. 5:

5. Copper (Inv. TA-201-32)—Vote on injury (at 2 p.m.) *and briefing on remedy, if necessary.*

CONTACT PERSON FOR MORE INFORMATION:

Kenneth R. Mason, Secretary, 202-523-0161.

[S-1554-78 Filed 7-26-78; 3:54 pm]

[7020-02]

12

[USITC SE-78-37]

UNITED STATES INTERNATIONAL TRADE COMMISSION.

TIME AND DATE: 10 a.m., Thursday, August 10, 1978.

PLACE: Room 117, 701 E Street NW., Washington, D.C. 20436.

STATUS: Open to the public.

MATTERS TO BE CONSIDERED:

1. Agenda.
2. Minutes.
3. Ratifications.
4. Petitions and complaints (if necessary).
5. Copper (Inv. TA-201-32)—Vote on remedy, if necessary.
6. Bicycle tires and tubes (Inv. TA-201-33)—Vote on injury.
7. Appeal of denial of information under the FOIA (if necessary).
8. Any items left over from previous agenda.

CONTACT PERSON FOR MORE INFORMATION:

Kenneth R. Mason, Secretary, 202-523-0161.

[S-1555-78 Filed 7-26-78; 3:54 pm]

[4110-39]

13

NATIONAL COUNCIL ON EDUCATIONAL RESEARCH.

The National Council on Educational Research hereby gives notice that it has tentatively scheduled meetings to be held in Washington, D.C., on the following dates: September 15, 1978; November 3, 1978; January 11-12, 1979; March 23, 1979; May 11, 1979; July 12-13, 1979.

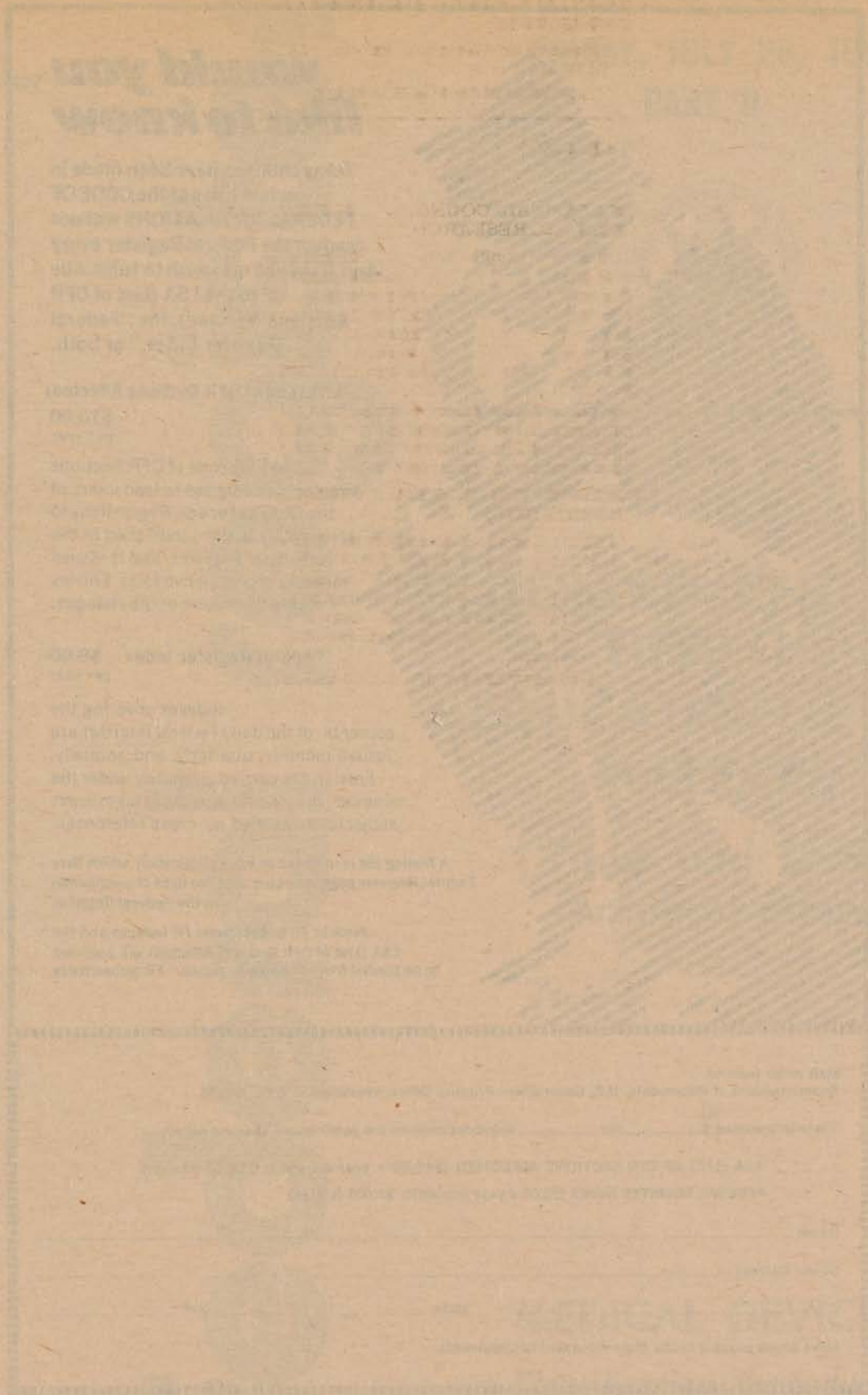
Agendas for these meetings and any changes in meeting dates or locations will be published in the FEDERAL REGISTER as promptly as possible.

PERSON TO CONTACT FOR INFORMATION:

Ella L. Jones, Administrative Coordinator, telephone 202-254-7900.

PETER H. GERBER,
Chief, Policy and Administrative Coordination, National Council on Educational Research.

[S-1548-78 Filed 7-26-78; 11:44 am]



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BOOK 2 OF 2 BOOKS

FRIDAY, JULY 28, 1978
PART II

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Food and Drug Administration

MEDICAL DEVICES

Classification Procedures

[4110-03]

Title 21—Food and Drugs

CHAPTER I—FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

SUBCHAPTER A—GENERAL

SUBCHAPTER H—MEDICAL DEVICES

[Docket No. 77N-0155]

Classification Procedures

AGENCY: Food and Drug Administration.

ACTION: Final rule.

SUMMARY: This rule sets forth criteria and procedures for classifying devices intended for human use into classes of regulatory control sufficient to provide reasonable assurance of safety and effectiveness. The rule also explains the determination of the safety and effectiveness of devices, prescribes the procedures for the submission and review of petitions for reclassification, and defines the circumstances under which information and data associated with the classification or reclassification of devices will be released to the public. These actions are taken under the Medical Device Amendments of 1976.

EFFECTIVE DATE: August 28, 1978.

FOR FURTHER INFORMATION CONTACT:

Joseph Sheehan, Bureau of Medical Devices (HFK-70), Food and Drug Administration, Department of Health, Education, and Welfare, 8757 Georgia Avenue, Silver Spring, Md. 20910, 301-427-7114.

SUPPLEMENTARY INFORMATION: The proposal upon which this final regulation is based was published in the FEDERAL REGISTER of September 13, 1977 (42 FR 46028). Interested persons were given until November 14, 1977 to comment. Twenty-five comments were received on the proposal, presenting a wide range of issues.

This regulation essentially codifies existing procedures that have been followed in the classification process to date. Manufacturers and other interested persons have already become involved in the various aspects of the classification process described in this regulation. The agency has been urged to promulgate this regulation as quickly as possible to provide industry a more certain basis for production decisions. Although the Commissioner doubts that this procedural regulation will affect manufacturers' production decisions, the Commissioner has decided that it is in the best interest of the public and of all parties concerned that this regulation become effective August 28, 1978.

GENERAL

The Commissioner has made many minor editorial changes in the regulation for clarity.

1. Several comments stated that publication of panel recommendations and proposed regulations for the classification of devices before promulgation of the final regulation establishing classification procedures violates the basic principles of administrative rulemaking. The comments pointed out that an agency must provide public notice and an opportunity for interested parties to participate before implementation of a rule.

Section 513(c)(1) of the act requires the promulgation by regulation of the procedures to be followed by classification panels in making their reviews and recommendations. The section does not require, however, that the final classification procedures regulation precede every other step in the classification process. Moreover, this classification procedures regulation essentially codifies the procedures that the agency has been following in the classification process. Public notice of these procedures was provided in a notice published in the FEDERAL REGISTER on May 19, 1975 (40 FR 21848). Because classification panels are public advisory committees, the general procedures under which the panels operate have already been promulgated by regulation (21 CFR Part 14).

2. One comment, referring to the portion of the preamble to the proposed regulation that discussed the classification criteria (42 FR 46030), argued against consideration of such "practical matters" as the difficulty involved in enforcing general controls and the length of time required to develop performance standards. The comment stated that such considerations should be irrelevant to classification decisions, and that any inconvenience to the agency does not change the fact that adequate information may exist to allow proper classification in accordance with the statutory criteria.

The Commissioner agrees that it is improper to consider the length of time required to develop a performance standard when determining whether to classify a device into class II unless compliance with a standard is essential to provide reasonable assurance of a device's safety and effectiveness. The legislative history reveals both that Congress recognized that considerable time may elapse between classification of a device into class II and the development of a performance standard for the device (Ref. 1, p. 27), and that FDA has ample latitude to classify a device into the premarket approval category in instances in which use of the device poses public health concerns. The Commissioner believes also, however, that the degree of difficulty involved in enforcing general controls with respect to a particular device may well be a relevant consideration in determining whether general controls will provide reasonable assurance of the safety and effectiveness of the device.

3. A few comments expressed concern that the definition of "implant" in proposed § 860.3(d) would include many devices which should not be classified into class III, such as dental fillings. The comments suggested that the proposed definition be worded so as not to include such devices.

The Commissioner acknowledges the broad scope of the proposed definition, but also notes that a device which is termed an implant is not necessarily classified into class III. Sections 513 (c)(2)(C) and (d)(2)(B) of the act clearly states that an implant need not be classified into class III if such classification is not necessary to provide reasonable assurance of safety and effectiveness. The proposed definition, therefore, has been retained without change in the final regulation.

4. Several comments requested revision of the proposed definition of "life-supporting or life-sustaining device" in § 860.3(e). The comment suggested that the proposed wording is redundant and vague. The comments also stated that the proposed definition is too broad because Congress intended that only devices essential to supporting or sustaining life be considered life-supporting or life-sustaining devices for classification purposes. Some comments suggested that the words "or yields information that

is to be used for restoration, maintenance or continuation of such function" be deleted from the definition. Other comments suggested that the definition be reworded to include only devices the discontinuance of which would result in a high probability of death.

The proposed definition has been reworded. The Commissioner believes that the special regulatory treatment afforded life-supporting or life-sustaining devices is necessary for devices which yield information essential to supporting or sustaining life, as well as for devices which are themselves life-supporting or life-sustaining. The Commissioner also rejects the idea that discontinuation of the use of a particular device must result in a high probability of death in order for that device to be properly termed life-supporting or life-sustaining. Congress expressed its intent that the phrase "life-supporting or life-sustaining" be interpreted broadly (ref. 1, p. 35). The Commissioner has reworded the definition to eliminate redundancy and to reflect more accurately the congressional intent (ref. 2, p. 58).

5. A few comments objected to the fact that the classification questionnaire was included in the preamble but not in the proposed regulation. The comments stated that the questionnaire is a substantive part of the classification process and expressed concern that if it were not included in the regulation FDA could revise the questionnaire without notice.

The classification questionnaire is merely a guideline intended to aid the panels in applying the legal requirements to the practical task of device classification. Devices will be classified and reclassified only according to the criteria in section 513 of the act. The entire questionnaire may not be applicable to all present cases. Furthermore, it is foreseeable that technological developments or other circumstances might necessitate future changes in the questionnaire, although such significant changes should be rare and would be announced by appropriate notice published in the FEDERAL REGISTER. Copies of the current classification questionnaire may be obtained from the Classification Coordinator (HFK-401), Bureau of Medical Devices, Food and Drug Administration, 8757 Georgia Avenue, Silver Spring, Md. 20910.

6. Some comments suggested that § 860.3(g)(7), requiring the supplemental data sheet to identify any needed restrictions on the use of the device, exceeds classification panel authority and requires a level of experience and competence beyond that possessed by most classification panel members. The comments suggested that because the power to recommend restrictions on the use of a device could be abused, and because of possible conflicts of interest, any identification of necessary restrictions should be accompanied by a statement of reasons and should be supported by objective evidence. Several comments also recommended that the phrase "authorization of a physician" be changed to "authorization of a licensed practitioner," to conform with the wording of section 520(e) of the act.

The general classification recommendations, required to be made by classification panels, necessarily involve identification of any needed restrictions on the use of the device. Moreover, there is no prohibition against FDA's asking its classification panels for advice on needed restrictions on device use or other matters within FDA's authority, even if the act does not require FDA to solicit such advice. The Commissioner rejects the contention that such determinations are beyond the authority or competence of the panels, or that they present the panels with a unique opportunity to abuse their power. These objections have been adequately considered and provided for in the act and in FDA regulations. The restrictions mentioned in § 860.3(g)(7) are merely examples of the possible types of restrictions on the use of a device which may be necessary. The phrase "authorization of a physician" has been changed in the final regulation to "authorization of a licensed practitioner" to reflect the wording in section 520(e) of the act.

7. Several comments on proposed § 860.3(i) questioned the need to introduce the new term "generic type of device." The comments suggested that the statutory terms "within a type" and "substantially equivalent" are accepted and well understood, while the phrase "generic type of device" is vague.

The Commissioner rejects the suggestion that the phrase "generic type of device" is vague and is at variance with the statute. The statutory terms, "within a type" and "substantially equivalent," serve a purpose different from that of FDA's definition of "generic type of device." Application of the two statutory terms determines whether a device is classified in class III under sections 513(f) and 520(l)(1)(D) of the act. The term "generic type of device" describes FDA's grouping, for reasons of administrative convenience, of devices that are to be regulated in the same way because they present similar safety and effectiveness concerns. A generic type of device will include devices that may or may not be "within a type" and "substantially equivalent" to each other.

For example, to reduce unnecessary proliferation of regulations, FDA may treat all pathology stains as a single generic type of device and may issue a single proposed and final classification regulation concerning these stains. If a single regulation is issued, not all of the stains in the generic type, and covered by the regulation, would be "substantially equivalent" to each other. If a manufacturer submitted a premarket notification seeking to market a new stain, FDA would determine whether the new stain was "substantially equivalent" to any stain in the generic type, that is subject to the classification regulation, and thus whether the new stain was itself subject to the regulation. If the new stain was not "substantially equivalent" to any stain covered by the regulation, the new stain would be classified into class III by section 513(f) of the act. If the manufacturer sought reclassification of the new stain, under § 860.120(b) only the new stain and any later "substantially equivalent" devices would be affected by the reclassification.

The Commissioner warns that FDA may find it difficult to describe the generic type of devices subject to a classification regulation so precisely that an interested person will be able to determine whether a new device is subject to the regulation merely by reading it, without FDA's comparing the new device to existing devices already covered by the regulation. This difficulty is recognized and addressed in the premarket notification procedure in section 510(k) of the act and 21 CFR 807.81.

8. One comment pointed out that the requirement of proposed § 860.5(c)(1) that safety and effectiveness data regarding devices classified into class III be regarded as confidential and not be disclosed unless the data have been disclosed previously to the public was impossible to satisfy under the present system of open panel meetings. The comment suggested that some provision be made for closed panel meetings.

The requirements of 21 CFR 14.27, which are applicable to classification panel meetings, provide that portions of a panel meeting may be closed for discussion of certain matters including trade secrets and confidential commercial information.

9. The comments on proposed § 860.5 (d) and (e) questioned the legality of publicly disclosing otherwise confidential information when contain in reclassification petitions. The comments suggested several provisions which would restrict the extent to which such information would be subject to public disclosure. The comments also questioned the apparent inconsistency in proposed § 860.5(d)(2) which allows a petitioner to show that any of the contents of a deficient petition should

be exempt from public disclosure, when the entire contents of the petition are disclosable once the deficiencies have been corrected.

The Commissioner has determined that a petitioner voluntarily surrenders the confidentiality of the contents of a nondeficient reclassification petition. As explained in the preamble to the proposed regulation, the loss of confidentiality is based in part on the necessity that reclassification proceedings be as open as possible. Because all devices within a generic type are reclassified together, one reclassification petition may affect several manufacturers, and each manufacturer affected by the petition should be afforded an opportunity to address the issue.

Because reclassification decisions concern generic types of devices, trade secrets, or other confidential information which relates only to individual devices is irrelevant to reclassification decisions. The Commissioner neither requires nor desires that manufacturers or importers submit such confidential information in reclassification petitions. Furthermore, the Commissioner has determined that the legislative intent precludes the use of such confidential information as the basis for the reclassification of devices into class III (ref. 1, pp. 48 through 50). See section 520(c) of the act (21 U.S.C. 360j(c)). Consequently, petitioners should not include in their petitions any data or information that is unnecessary to a decision on the petition, especially if the petitioner wishes to keep such data or information confidential.

Because the policy of disclosure is not yet widely known by those petitioning for reclassification, all reclassification petitions will be screened for possible confidential information at the same time that they are being reviewed for deficiencies, until 180 days after the final classification regulation becomes effective. All petitioners will be offered an informal opportunity to delete any confidential data from their petition, or to withdraw the entire petition before it becomes available for public disclosure. However, 180 days after the final classification regulation becomes effective, petitioners should have become familiar with the reclassification process, and FDA will cease reviewing nondeficient petitions for confidential information and will make the entire contents of all petitions available for public disclosure once they have been determined to be nondeficient.

Because a deficient petition will not be considered on its merits until the deficiencies have been corrected, submission of a petition is not considered a surrender of the confidentiality of its contents until all deficiencies have been corrected. Following notification of a deficiency in the petition, the petitioner is allowed a period of time in which to submit supplemental material intended to correct the deficiency. To provide adequate time for response to a notification of deficiency, the Commissioner has increased the period of time allowed from 20 days to 30 days. If, during this 30-day period, the petitioner wishes to withdraw the deficient petition rather than have its contents be available to the public, the Commissioner, in his discretion, may allow the withdrawal. This provision has been added to §860.5(d)(2). Once the deficient petition has been corrected, the entire contents of the petition will be available for public disclosure. The Commissioner has added the provision that any supplemental material submitted by the petitioner, together with the material in the original petition, is considered as a new petition. The new petition is reviewed for deficiencies in the same manner as the original petition, and the same procedures for notification and correction of deficiencies are followed. The Commissioner has also added the provision that a deficient petition which is not corrected within 180 days after notification of a deficiency will be returned to the petitioner, and will not be considered further unless resubmitted.

10. One comment stated that the provisions of §860.7, defining valid scientific evidence and well-controlled investigations should apply only to evidence developed or compiled after the effective date of the final regulation.

The provisions in that section do not depart from traditionally recognized concepts, are flexible, are required by section 513(a)(3) of the act, and closely reflect the legislative intent in this area (ref. 1, pp. 17 and 40). Because the provisions do not require a new approach to the proper substantiation of device safety or effectiveness, there is no valid reason for their being applied only to evidence compiled or developed after the effective date of the final regulation.

The Commissioner has deleted the first sentence of §860.7(a) because it is obvious that no single standard of safety and effectiveness could apply to all devices, and it is not necessary to include that fact in the regulation.

11. Several comments addressed the safety and effectiveness factors listed in proposed §860.7(b). The comments suggested that advertising should not be considered with regard to intended conditions of use, that surgical risks should not be considered when weighing the risks of implants, and that the reliability of a device is not a relevant factor in determining its safetly and effectiveness. Several comments also suggested that classification panels should either include engineers as members or seek technical engineering advice if the reliability of devices must be considered.

The legislative history of section 513 of the act clearly reveals that Congress intended the phrase "conditions of use" to include uses promoted through advertising, but that a device should not be regarded as unsafe merely because of "collateral risks" not inherent in the use of the device (ref. 1, p. 16). The Commissioner believes that FDA must retain some discretion in determining which surgical risks are to be considered inherent in the use of any particular device, including implants. The legislative history also reveals that Congress intended that device reliability be considered in determining device safety and effectiveness (ref. 1, p. 16). Furthermore, engineers are represented on panels in order to facilitate consideration of device reliability. Consequently, §860.7(b) has been retained without change in the final regulation.

12. Several comments argued that proposed §860.7(c), by restricting consideration to valid scientific evidence when determining the safety and effectiveness of a device, does not reflect accurately the legislative intent. Several comments also questioned whether the Commissioner should have the authority, "in his discretion," to determine whether evidence submitted is valid scientific evidence. Several comments also suggested that if nonvalid scientific evidence is irrelevant in establishing the effectiveness of a device, such evidence also should be irrelevant in establishing that a device is not effective. One comment suggested that the panel should investigate and corroborate islolated case reports, random experience, and similar forms of evidence.

The purpose of the act is to assure the safety and effectiveness of medical devices intended for human use. Because such assurance necessarily demands a high standard of proof, section 513(a)(3) of the act requires that device effectiveness be established only by valid scientific evidence. The Commissioner has extended this requirement to the establishment of device safety as well. The requirement that only valid scientific evidence be used to establish device safety and effectiveness, however, does not preclude consideration of other forms of evidence when determining whether a device is safe or effective. Although it is imperative that early and sometimes informal indications of the danger or ineffectiveness of a device be considered fully, every effort will be made to corroborate such evidence before acting on it. The phrase "in his discretion" has been deleted from the second sentence of §860.7(c)(1) in the final regulation because it is superflu-

ous, but the remainder of the section has not been changed.

13. Comments on proposed § 860.7(d) requested a more objective and specific definition of what constitutes reasonable assurance of device safety, and clearer guidelines as to what constitutes "adequate efforts to demonstrate the absence of unreasonable risk of illness or injury."

The wording of the proposed section closely follows the wording of section 513 of the act. The legislative history (ref. 1, pp. 16-17) explains that determination of device safety involves balancing the probable benefits of a device against its probable risks. Consequently, proof of device safety is intended to establish that the risks are not unreasonably disproportionate to the benefits. The proposed section merely expands this concept, emphasizing that only valid scientific evidence may be used to establish device safety. The Commissioner does not believe that any change in the final regulation is necessary.

14. One comment on proposed § 860.7(e) suggested that a determination of device effectiveness should be based upon whether a device meets the claims of its manufacturer.

Section 513 of the act and § 860.7(b)(2) provide that the effect which a device purports or is represented to have is to be considered in determining its effectiveness. Proposed § 860.7(e) is consistent with those provisions and has been retained in the final regulation with minor clarifying word changes.

A question has arisen concerning the number of studies required to establish the effectiveness of a device. The Commissioner advises that section 513(a)(3) of the act and § 860.7 (e) and (f) require at least two well-controlled investigations showing effectiveness, unless the Commissioner, under § 860.7(f), authorizes reliance upon other valid scientific evidence. With respect to the general requirement of two or more effectiveness studies, device law is similar to drug law (sec. 505(d) of the act, 21 U.S.C. 355(d); 21 CFR 312.1, 314.111(a)(5)).

15. Comments suggested that the testing described in proposed § 860.7(f) is oriented too heavily toward drugs and is not appropriate for electronic, mechanical, or similar devices. It was suggested that many of these devices could be adequately tested by purely electronic or similar testing.

Section 860.7(e) provides that other valid scientific evidence may be used to prove device effectiveness in cases where the Commissioner determines that the requirements of § 860.7(f) are not reasonably applicable or essential to the testing of the device in question. Furthermore, the requirements of § 860.7(f) are designed to allow some leeway, including alternative approaches in some areas, and thus should prove compatible with most device testing.

16. Comments objected to proposed § 860.7(g) which requires the manufacturer or importer to show that general controls or performance standards would provide sufficient assurance of the safety and effectiveness of a device. The comments suggested that failure to make such a showing should not result in classification of the device into class III if the device is of the type described in section 513(a)(1)(A)(ii) of the act. Other comments suggested that the section be deleted in its entirety. Several comments also objected to the provision authorizing the Commissioner to require device manufacturers, importers, or distributors to submit reports or other information. The comments objected that, because section 519 of the act contemplated a more specific rulemaking process, such reporting requirements may not be promulgated in this manner.

The legislative history indicates that Congress intentionally placed upon industry the burden of furnishing sufficient evidence to substantiate the safety and effectiveness of a device, and that the absence of such data may be the basis for classification of the device into class III (ref. 1, p. 40). Although section 513(a)(1)(A)(ii) of the act provides that some devices may be classified into class I even if there is insufficient information from which to determine that general controls would provide reasonable assurance of their safety and effectiveness, such classification is permitted only for devices which do not present a potential unreasonable risk of illness or injury. In the absence of safety and effectiveness data, it may be impossible to determine that no such potential risk exists. The Commissioner emphasizes the need for industry to provide collectively sufficient safety and effectiveness data to ensure the proper classification of each generic type of device. The Commissioner believes that § 860.7(g) complies with the requirements of section 519 of the act.

CLASSIFICATION

17. One comment on proposed § 860.84 suggested that a classification of a device by the Commissioner which differs from the classification recommended by the panel should be supported by valid scientific evidence. It was also suggested that panel recommendations be required to identify only unreasonable risks to health, rather than all risks to health, presented by a particular device.

The act clearly provides the basis for determining device safety and effectiveness and prescribes the criteria for device classification. All classification decisions reached by the Commissioner or by the classification panels will be made in accordance with the provisions of the act. The Commissioner may disagree with a panel recommendation if his interpretation of available scientific evidence differs from that of the panel. In addition, the Commissioner intends to evaluate carefully panel recommendations in istances where valid scientific evidence is not available to support a panel recommendation. The requirement that panel recommendations specifically include identification of the risks to health presented by a device is based on section 513(c)(2)(A)(i)(III) of the act.

The Commissioner has redesignated some of the paragraphs in proposed § 860.84 in order to facilitate reference, but the section has been incorporated in the final regulation with no substantive changes.

18. One comment on proposed § 860.95 suggested that, in their recommendations, panels should not be required to state the reasons for recommending that a device be exempt from the requirements of section 510, 519, or 520(f) of the act. Another comment suggested that a regulation or order classifying or reclassifying a device into class I be required to state the reasons for not granting exemptions as well as the reasons for granting exemptions.

Section 513 of the act requires that panel recommendations for classification into class I, and final regulations or orders classifying devices into class I, specify whether the device is exempted from the requirements of sections 510, 519, or 520(f) of the act. The legislative history reveals that Congress considered "general controls," such as those provided for in sections 510, 519, and 520(f), important safeguards for public health (ref. 1, p. 17). Consequently, the act provides for exemption from only certain general controls, and requires that such exemptions be justified. There is no need to justify a requirement that devices that are not exempted comply with general controls. Proposed § 860.95 has been incorporated in the final regulation without change.

RECLASSIFICATION

19. One comment on proposed § 860.120 suggested that reclassification of one device within a generic type of device should not cause reclassification of all other devices within that generic type unless all such devices present the same unreasonable risk. Another comment stated that some clarification was needed as to who may file a reclassification petition. Several comments suggested that manufacturers and importers who

have not petitioned for the reclassification of a device should be provided a reasonable time to comply with new requirements applicable to the device following its reclassification.

By definition, all devices within a generic type present the same or very similar risks to health. The similarity in health risks is fundamental to the concept of classification by generic type of device. If devices thought to be within the same generic type present different risks, it is likely that the devices are not really of the same generic type. Manufacturers and importers of devices within a generic type will be provided an opportunity to participate in all reclassification proceedings regarding the generic type of device. The open nature of the reclassification process is assured by the provisions in the regulation regarding public disclosure of reclassification of petitions and panel recommendations. If compliance with a performance standard is required because of reclassification, the performance standard will be promulgated under the procedures in section 514 of the act which provide manufacturers notice and a grace period for compliance.

The Commissioner has added new § 860.120(c) to clarify who may file a petition for reclassification. FDA previously announced, in 21 CFR 10.25, its general policy that any interested party (whether a manufacturer, consumer, importer, or member of the public) may petition the Commissioner to issue, amend, or revoke a regulation or order promulgated by him. As explained below, reclassification petitions are subject to the special procedures of subpart C of part 860 rather than the citizen petition procedures. However, under the policy of the citizen petition procedure as applied to the classification process, any interested person is afforded an opportunity to file a petition for reclassification under the regulation based on sections 513(e), 514(b), or 515(b) of the act. The reclassification process under section 513(f) or 520(1) of the act is limited to the manufacturer or importer of the specific device involved because of the special procedural safeguards for reclassifying new devices and devices previously regarded as new drugs. The Commissioner has reorganized the remainder of proposed § 860.120 for clarification, but the substance of the section has not been changed in the final regulation.

20. Two comments on proposed § 860.123 questioned why reclassification petitions were not treated as citizen petitions, and why the Commissioner was not required to respond to such petitions within a definite period of time.

Section 10.30 (21 CFR 10.30) defines "citizen petitions" and provides that other sections regarding other types of petitions may include different requirements. The Commissioner has determined that petitions for device reclassification shall conform to the requirements of proposed § 860.123, and has added new § 860.3(j) to define the meaning of the term "petition" as it is used in part 860. The Commissioner agrees that a response time should be provided for reclassification petitions submitted under section 513(e) of the act, and § 860.130 of the final regulation has been revised accordingly. The act provides specific response times for all other reclassification petitions.

21. Several comments on proposed § 860.125 objected that consultation by the Commissioner with less than an entire panel would defeat the effectiveness of the panel and is contrary to the legislative intent in this area. It was also mentioned that informal means of consultation, especially consultation by telephone, might result in an unsatisfactory record. One comment suggested that a petitioner should be able to request that the Commissioner consult with the classification panel at a regular panel meeting.

The Commissioner agrees that every effort should be made to consult with an entire classification panel, and that an adequate record of such consultation is essential. There will be circumstances, however, in which statutory time constraints, the request by the petitioner for a timely response, or the unavailability of panel members will require the Commissioner to consult with less than an entire panel, and by means other than discussion at a regular panel meeting. The Commissioner had preceded the phrase "a majority of current voting panel members," in proposed § 860.125(a) (1) and (2), with the words "at least." Whenever possible, the Commissioner will consult with nonvoting members, and § 860.125(a) (1) and (2) has been changed accordingly.

22. Comments on proposed § 860.130 suggested that the Commissioner be required to secure a panel recommendation for reclassification of a device under section 513(e), that all reclassifications of devices from class III to class II should take effect immediately, that all regulations promulgating reclassifications should identify and revoke specific requirements of the prior classification which are no longer applicable to the device, and that the Commissioner should be required to publish oral panel recommendations made under § 860.125(a) (1) or (3) as well as written recommendations. One comment also noted that no deadline is provided for response to petitions submitted under this section.

Section 513(e) of the act provides that the Commissioner may act on the basis of new information to reclassify a device without seeking a panel recommendation. There will be circumstances in which the Commissioner will need to consult with a panel in order to reach a proper decision regarding such reclassifications. Whenever a panel is consulted, any panel advice will be recorded, whether the advice is written or oral. Oral advice will be written down. A regulation reclassifying a device will identify and revoke all requirements of the previous classification which no longer apply to the device. In the case of devices reclassified from class III to class II, section 513(e) of the act specifically provides that the effective date of a reclassification in class II may be delayed pending the development of a performance standard for the device, and FDA will take this approach when it is appropriate. The Commissioner has added the provision that petitions submitted under § 860.130 will be approved or denied within 180 days after the filing of the petition.

23. One comment on proposed § 860.132 suggested that the title be reworded, and that the text of § 860.132(b) be condensed and reorganized. The proposed title of § 860.132 was "Procedures when the Commissioner initiates a performance standard or premarket approval requirement under section 514(b) or 515(b) of the act." Several comments also objected that the 15-day deadline for filing petitions is inadequate.

The Commissioner believes that the title of proposed § 860.132, when read in the context of the other sections in the reclassification subpart, clearly states the subject of the section. The Commissioner recognizes no need to reorganize the section. Sections 514(b) and 515(b) of the act require the 15-day deadline for the submission of a petition. Proposed § 860.132 has been incorporated in the final regulation with minor editorial changes.

24. Comments on proposed § 860.134 suggested that the 210-day response time was too long, that an order denying a petition should set forth the reasons for the denial, that any decision by the Commissioner which differs form the panel recommendation should be supported by valid scientific evidence, and that any interested person should be able to petition for reclassification under this section.

The 210-day period for final action on reclassification petitions for "new devices" is established by section 513(f) of the act and is not unreasonable in light of the many steps required to process such petitions. Any order denying a petition for reclassification of a new device will set forth the reasons for that decision, as will orders approving such petitions. The Commissioner's decision regarding re-

classification will be based upon the same criteria considered by panels in making their recommendations, and will be in accordance with the provisions of the act. Section 513(f) of the act authorizes only the manufacturer or importer of a "new device" to initiate reclassification proceedings for the device. Other interested persons may seek reclassification of the device under section 513(e) of the act and § 860.130. The Commissioner has made minor changes in the wording of the proposed section in order to follow more closely the wording of section 513(f) of the act.

REFERENCES

Background data and information upon which the Commissioner relies in promulgating this regulation have been placed on file for public review in the office of the Hearing Clerk (HFC-20), Food and Drug Administration, Room 4-65, 5600 Fishers Lane, Rockville, Md. 20857. The following is a list of those documents:

1. House Report No. 94-853, Medical Device Amendments, February 29, 1976 (Committee on Interstate and Foreign Commerce).

2. House Report No. 94-1090, Medical Device Amendments, May 6, 1976 (Committee of Conference).

Therefore, under the Federal Food, Drug, and Cosmetic Act (secs. 513, 514, 515, 519, 520, and 701(a), 52 Stat. 1055, 90 Stat. 540-559, 564-574 (21 U.S.C. 360c, 360d, 360e, 360i, 360j, and 371(a))), and under authority delegated to the Commissioner (21 CFR 5.1), 21 CFR Chapter I is amended as follows:

PART 16—REGULATORY HEARING BEFORE THE FOOD AND DRUG ADMINISTRATION

1. Part 16 is amended in § 16.1 by adding new paragraph (b)(31), to read as follows:

§ 16.1 Scope.

* * * * *

(b) * * *

(31) Section 860.136 of this chapter, relating to petitions for reclassification of a medical device currently in class III by operation of section 520(l)(1) of the Federal Food, Drug, and Cosmetic Act.

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PART 20—PUBLIC INFORMATION

2. Part 20 is amended in § 20.100 by adding new paragraph (c)(31), to read as follows:

§ 20.100 Applicability; cross-reference to other regulations.

* * * * *

(c) * * *

(31) Data and information submitted to the Commissioner or to classification panels in connection with the classification or reclassification of devices intended for human use, in § 860.5 of this chapter.

3. Part 860 is added to read as follows:

PART 860—MEDICAL DEVICE CLASSIFICATION PROCEDURES

Subpart A—General

Sec.
860.1 Scope.
860.3 Definitions.
860.5 Confidentiality and use of data and information submitted in connection with classification and reclassification.
860.7 Determination of safety and effectiveness.

Subpart B—Classification

860.84 Classification procedures for "old devices."
860.93 Classification of implants, life-supporting or life-sustaining devices.
860.95 Exemptions from sections 510, 519, and 520(f) of the act.

Subpart C—Reclassification

860.120 General.
860.123 Reclassification petition: content and form.
860.125 Consultation with panels.
860.130 General procedures under section 513(e) of the act.
860.132 Procedures when the Commissioner initiates a performance standard or premarket approval proceeding under section 514(b) or 515(b) of the act.
860.134 Procedures for "new devices" under section 513(f) of the act.
860.136 Procedures for transitional products under section 520(l) of the act.

AUTHORITY: Secs. 513, 514, 515, 519, 520, and 701(a), 52 Stat. 1055, 90 Stat. 540-559, 564-574 (21 U.S.C. 360c, 360d, 360e, 360i, 360j, and 371(a)), unless otherwise noted.

Subpart A—General

§ 860.1 Scope.

(a) This part implements sections 513, 514(b), 515(b), and 520(l) of the act with respect to the classification and reclassification of devices intended for human use.

(b) This part prescribes the criteria and procedures to be used by classification panels in making their recommendations and by the Commissioner in making the Commissioner's determinations regarding the class of regulatory control (class I, class II, or class III) appropriate for particular devices. Supplementing the general Food and Drug Administration procedures governing advisory committees (part 14 of this chapter), this part also provides procedures for manufacturers, importers, and other interested persons to participate in proceedings to classify and reclassify devices. This part also describes the kind of data required for determination of the safety and effectiveness of a device, and the circumstances under which information submitted to classification panels or to the Commissioner in connection with classification and reclassification proceedings will be available to the public.

§ 860.3 Definitions.

For the purposes of this part:

(a) "Act" means the Federal Food, Drug, and Cosmetic Act.

(b) "Commissioner" means the Commissioner of Food and Drugs, Food and Drug Administration, United States Department of Health, Education, and Welfare, or the Commissioner's designee.

(c) "Class" means one of the three categories of regulatory control for medical devices, defined below:

(1) "Class I" means the class of devices that are subject to only the general controls authorized by or under sections 501 (adulteration), 502 (misbranding), 510 (registration), 516 (banned devices), 518 (notification and other remedies), 519 (records and reports), and 520 (general provisions) of the act. A device is in class I if (i) general controls are sufficient to provide reasonable assurance of the safety and effectiveness of the device, or (ii) there is insufficient information from which to determine that general controls are sufficient to provide reasonable assurance of the safety and effectiveness of the device or to establish a performance standard to provide such assurance, but which is not life-supporting or life-sustaining or for a use which is of substanial importance in preventing impairment of human health, and which does not present a potential unreasonable risk of illness of injury.

(2) "Class II" means the class of devices that are or eventually will be subject to the requirements of a performance standard promulgated in accordance with section 514 of the act. A device is in class II if general controls alone are insufficient to provide reasonable assurance of its safety and effectiveness and there is sufficient information to establish a performance standard to provide such assurance.

(3) "Class III" means the class of devices for which premarket approval is or will be required in accordance with section 515 of the act. A device is in class III if insufficient information exists to determine that general controls are sufficient to provide reasonable assurance of its safety and effectiveness or to establish a performance standard to provide such assurance and if, in addition, the device is life-

supporting of life-sustaining, or for a use which is of substantial importance in preventing impairment of human health, or if the device presents a potential unreasonable risk of illness or injury.

(d) "Implant" means a device that is placed into a surgically or naturally formed cavity of the human body. A device is regarded as an implant for the purpose of this part only if it is intended to remain implanted continuously for a period of 30 days or more, unless the Commissioner determines otherwise in order to protect human health.

"Life-supporting or life-sustaining device" means a device that is essential to, or that yields information that is essential to, the restoration or continuation of a bodily function important to the continuation of human life.

(f) "Classification questionnaire" means a specific series of questions prepared by the Commissioner for use as guidelines by classification panels preparing recommendations to the Commissioner regarding classification and by petitioners submitting petitions for reclassification. The questions relate to the safety and effectiveness characteristics of a device and the answers are designed to help the Commissioner determine the proper classification of the device.

(g) "Supplemental data sheet" means information compiled by a classification panel or submitted in a petition for reclassification, including:

(1) A summary of the reasons for the recommendation (or petition);

(2) A summary of the data upon which the recommendation (or petition) is based;

(3) An identification of the risks to health (if any) presented by the device;

(4) To the extent practicable in the case of a class II or class III device, a recommendation for the assignment of a priority for the application of the requirements of performance standards or premarket approval;

(5) In the case of a class I device, a recommendation whether the device should be exempted from any of the requirements of registration, recordkeeping and reporting, or good manufacturing practice regulations;

(6) In the case of an implant or a life-supporting or life-sustaining device for which classification in class III is not recommended, a statement of the reasons for not recommending that the device be classified in class III;

(7) Identification of any needed restrictions on the use of the device, e.g., whether the device requires special labeling, should be banned, or should be used only upon authorization of a practitioner licensed by law to administer or use such device; and

(8) Any known existing standards applicable to the device, device components, or device materials.

(h) "Classification panel" means one of the several advisory committees established by the Commissioner under section 513 of the act and part 14 of this chapter for the purpose of making recommendations to the Commissioner on the classification and reclassification of devices and for other purposes prescribed by the act or by the Commissioner.

(i) "Generic type of device" means a grouping of devices that do not differ significantly in purpose, design, materials, energy source, function, or any other feature related to safety and effectiveness, and for which similar regulatory controls are sufficient to provide reasonable assurance of safety and effectiveness.

(j) "Petition" means a submission seeking reclassification of a device in accordance with § 860.123.

§ 860.5 Confidentiality and use of data and information submitted in connection with classification and reclassification.

(a) This section governs the availability for public disclosure and the use by the Commissioner of data and information submitted to classification panels or to the Commissioner in connection with the classification or reclassification of devices under this part.

(b) In general, data and information submitted to classification panels in connection with the classification of devices under § 860.84 will be available immediately for public disclosure upon request. However, except as provided by the special rules in paragraph (c) of this section, this provision does not apply to data and information exempt from public disclosure in accordance with part 20 of this chapter: Such data and information will be available only in accordance with part 20.

(c)(1) Safety and effectiveness data submitted to classification panels or to the Commissioner in connection with the classification of a device under § 860.84, which have not been disclosed previously to the public, as described in § 20.81 of this chapter, shall be regarded as confidential if the device is classified in to class III. Because the classification of a device under § 860.84 may be ascertained only upon publication of a final regulation, all safety and effectiveness data that have not been disclosed previously are not available for public disclosure unless and until the device is classified into class I or II, in which case the procedure in paragraph (c)(2) of this section applies.

(2) Thirty days after publication of a final regulation under § 860.84 classifying a device into class I or class II, safety and effectiveness data submitted for that device that had been regarded as confidential under paragraph (c)(1) of this section will be available for public disclosure and placed on public display in the office of the Hearing Clerk, Food and Drug Administration unless, within that 30-day period, the person who submitted the data demonstrates that the data still fall within the exemption for trade secrets and confidential commercial information described in § 20.61 of this chapter. Safety and effectiveness data submitted for a device that is classified into class III by regulation in accordance with § 860.84 will remain confidential and unavailable for public disclosure so long as such data have not been disclosed to the public as described in § 20.81 of this chapter.

(3) Because device classification affects generic types of devices, in making determinations under § 860.84 concerning the initial classification of a device, the classification panels and the Commissioner may consider safety and effectiveness data developed for another device in the same generic type, regardless of whether such data are regarded currently as confidential under paragraph (c)(1) of this section.

(d)(1) The fact of its existence and the contents of a petition for reclassification filed in accordance with § 860.130 or § 860.132 are available for public disclosure at the time the petition is received by the Food and Drug Administration.

(2) The fact of the existence of a petition for reclassification filed in accordance with § 860.134 or § 860.136 is available for public disclosure at the time the petition is received by the Food and Drug Administration. The contents of such a petition are not available for public disclosure for the period of time following its receipt (not longer than 30 days) during which the petition is reviewed for any deficiencies preventing the Commissioner from making a decision on it. Once it is determined that the petition contains no deficiencies preventing the Commissioner from making a decision on it, the petition will be filed with the Hearing Clerk and its entire contents will be available for public disclosure and subject to consideration by classification panels and by the Commissioner in making a decision on the petition. If, during this 30-day period of time, the petition is found to contain deficiencies that prevent the Commissioner from making a decision on it, the petitioner will be so notified and afforded an opportunity to correct the deficiencies.

Thirty days after notice to the petitioner of deficiencies in the petition,

the contents of the petition will be available for public disclosure unless, within that 30 days, the petitioner submits supplemental material intended to correct the deficiencies in the petition. The Commissioner, in the Commissioner's discretion, may allow withdrawal of a deficient petition during the 30-day period provided for correcting deficiencies. Any supplemental material submitted by the petitioner, together with the material in the original petition, is considered as a new petition. The new petition is reviewed for deficiencies in the same manner as the original petition, and the same procedures for notification and correction of deficiencies are followed. Once the petitioner has corrected the deficiencies, the entire contents of the petition will be available for public disclosure and subject to consideration by classification panels and by the Commissioner in making a decision on the petition. Deficient petitions which have not been corrected within 180 days after notification of deficiency will be returned to the petitioner and will not be considered further unless resubmitted.

(e) The Commissioner may not disclose, or use as the basis for reclassification of a device from class III to class II, any information reported to or otherwise obtained by the Commissioner under section 513, 514, 515, 516, 518, 519, 520(f), 520(g), or 704 of the act that falls within the exemption described in §20.61 of this chapter for trade secrets and confidential commercial information. The exemption described in §20.61 does not apply to data or information contained in a petition for reclassification submitted in accordance with §860.130 or §860.132, or in a petition submitted in accordance with §860.134 or §860.136 that has been determined to contain no deficiencies that prevent the Commissioner from making a decision on it. Accordingly, all data and information contained in such petitions may be disclosed by the Commissioner and used as the basis for reclassification of a device from class III to class II.

(f) For purposes of this section, safety and effectiveness data include data and results derived from all studies and tests of a device on animals and humans and from all studies and tests of the device itself intended to establish or determine its safety and effectiveness.

§860.7 Determination of safety and effectiveness.

(a) The classification panels, in reviewing evidence concerning the safety and effectiveness of a device and in preparing advice to the Commissioner, and the Commissioner, in making determinations concerning the safety and effectiveness of a device, will apply the rules in this section.

(b) In determining the safety and effectiveness of a device for purposes of classification, establishment of performance standards for class II devices, and premarket approval of class III devices, the Commissioner and the classification panels will consider the following, among other relevant factors:

(1) The persons for whose use the device is represented or intended;

(2) The conditions of use for the device, including conditions of use prescribed, recommended, or suggested in the labeling or advertising of the device, and other intended conditions of use;

(3) The probable benefit to health from the use of the device weighed against any probable injury or illness from such use; and

(4) The reliability of the device.

(c) (1) Although the manufacturer may submit any form of evidence to the Food and Drug Administration in an attempt to substantiate the safety and effectiveness of a device, the agency relies upon only valid scientific evidence to determine whether there is reasonable assurance that the device is safe and effective. After considering the nature of the device and the rules in this section, the Commissioner will determine whether the evidence submitted or otherwise available to the Commissioner is valid scientific evidence for the purpose of determining the safety or effectiveness of a particular device and whether the available evidence, when taken as a whole, is adequate to support a determination that there is reasonable assurance that the device is safe and effective for its conditions of use.

(2) Valid scientific evidence is evidence from well-controlled investigations, partially controlled studies, studies and objective trials without matched controls, well-documented case histories conducted by qualified experts, and reports of significant human experience with a marketed device, from which it can fairly and responsibly be concluded by qualified experts that there is reasonable assurance of the safety and effectiveness of a device under its conditions of use. The evidence required may vary according to the characteristics of the device, its conditions of use, the existence and adequacy of warnings and other restrictions, and the extent of experience with its use. Isolated case reports, random experience, reports lacking sufficient details to permit scientific evaluation, and unsubstantiated opinions are not regarded as valid scientific evidence to show safety or effectiveness. Such information may be considered, however, in identifying a device the safety and effectiveness of which is questionable.

(d) (1) There is reasonable assurance that a device is safe when it can be determined, based upon valid scientific evidence, that the probable benefits to health from use of the device for its intended uses and conditions of use, when accompanied by adequate directions and warnings against unsafe use, outweigh any probable risks. The valid scientific evidence used to determine the safety of a device shall adequately demonstrate the absence of unreasonable risk of illness or injury associated with the use of the device for its intended uses and conditions of use.

(2) Among the types of evidence that may be required, when appropriate, to determine that there is reasonable assurance that a device is safe are investigations using laboratory animals, investigations involving human subjects, and nonclinical investigations including in vitro studies.

(e) (1) There is reasonable assurance that a device is effective when it can be determined, based upon valid scientific evidence, that in a significant portion of the target population, the use of the device for its intended uses and conditions of use, when accompanied by adequate directions for use and warnings against unsafe use, will provide clinically significant results.

(2) The valid scientific evidence used to determine the effectiveness of a device shall consist principally of well-controlled investigations, as defined in paragraph (f) of this section, unless the Commissioner authorizes reliance upon other valid scientific evidence which the Commissioner has determined is sufficient evidence from which to determine the effectiveness of a device, even in the absence of well-controlled investigations. The Commissioner may make such a determination where the requirement of well-controlled investigations in paragraph (f) of this section is not reasonably applicable to the device.

(f) The following principles have been developed over a period of years and are recognized by the scientific community as the essentials of a well-controlled clinical investigation. They provide the basis for the Commissioner's determination whether there is reasonable assurance that a device is effective based upon well-controlled investigations and are also useful in assessing the weight to be given to other valid scientific evidence permitted under this section.

(1) The plan or protocol for the study and the report of the results of a well-controlled investigation shall include the following:

(i) A clear statement of the objectives of the study;

(ii) A method of selection of the subjects that:

(a) Provides adequate assurance that the subjects are suitable for the purposes of the study, provides diagnostic criteria of the condition to be treated or diagnosed, provides confirmatory laboratory tests where appropriate and, in the case of a device to prevent a disease or condition, provides evidence of susceptibility and exposure to the condition against which prophylaxis is desired;

(b) Assigns the subjects to test groups, if used, in such a way as to minimize any possible bias;

(c) Assures comparability between test groups and any control groups of pertinent variables such as sex, severity or duration of the disease, and use of therapy other than the test device;

(iii) An explanation of the methods of observation and recording of results utilized, including the variables measured, quantitation, assessment of any subject's response, and steps taken to minimize any possible bias of subjects and observers;

(iv) A comparison of the results of treatment or diagnosis with a control in such a fashion as to permit quantitative evaluation. The precise nature of the control must be specified and an explanation provided of the methods employed to minimize any possible bias of the observers and analysts of the data. Level and methods of "blinding," if appropriate and used, are to be documented. Generally, four types of comparisons are recognized:

(a) *No treatments.*—Where objective measurements of effectiveness are available and placebo effect is negligible, comparison of the objective results in comparable groups of treated and untreated patients;

(b) *Placebo control.*—Where there may be a placebo effect with the use of a device, comparison of the results of use of the device with an ineffective device used under conditions designed to resemble the conditions of use under investigation as far as possible;

(c) *Active treatment control.*—Where an effective regimen of therapy may be used for comparison, e.g., the condition being treated is such that the use of a placebo or the withholding of treatment would be inappropriate or contrary to the interest of the patient;

(d) *Historical control.*—In certain circumstances, such as those involving diseases with high and predictable mortality or signs and symptoms of predictable duration or severity, or in the case of prophylaxis where morbidity is predictable, the results of use of the device may be compared quantitatively with prior experience historically derived from the adequately documented natural history of the disease or condition in comparable patients or populations who received no treatment or who followed an established effective regimen (therapeutic, diagnostic, prophylactic).

(v) A summary of the methods of analysis and an evaluation of the data derived from the study, including any appropriate statistical methods utilized.

(2) To insure the reliability of the results of an investigation, a well-controlled investigation shall involve the use of a test device that is standardized in its composition or design and performance.

(g) (1) It is the responsibility of each manufacturer and importer of a device to assure that adequate, valid scientific evidence exists, and to furnish such evidence to the Food and Drug Administration to provide reasonable assurance that the device is safe and effective for its intended uses and conditions of use. The failure of a manufacturer or importer of a device to present to the Food and Drug Administration adequate, valid scientific evidence showing that there is reasonable assurance of the safety and effectiveness of the device, if regulated by general controls alone, or by general controls and performance standards, may support a determination that the device be classified into class III.

(2) The Commissioner may require that a manufacturer, importer, or distributor make reports or provide other information bearing on the classification of a device and indicating whether there is reasonable assurance of the safety and effectiveness of the device or whether it is adulterated or misbranded under the act.

(3) A requirement for a report or other information under this paragraph will comply with section 519 of the act. Accordingly, the requirement will state the reason or purpose for such request; will describe the required report or information as clearly as possible; will not be imposed on a manufacturer, importer, or distributor of a classified device that has been exempted from such a requirement in accordance with § 860.95; will prescribe the time for compliance with the requirement; and will prescribe the form and manner in which the report or information is to be provided.

(4) Required information that has been submitted previously to the Bureau of Medical Devices need not be resubmitted, but may be incorporated by reference.

Subpart B—Classification

§ 860.84 Classification procedures for "old devices."

(a) This subpart sets forth the procedures for the original classification of a device that either was in commercial distribution before May 28, 1976, or is substantially equivalent to a device that was in commercial distribution before that date. Such a device will be classified by regulation into either class I (general controls), class II (performance standards), or class III (premarket approval), depending upon the level of regulatory control required to provide reasonable assurance of the safety and effectiveness of the device (§ 860.3(c)). This subpart does not apply to a device that is classified into class III by statute under section 513(f) of the act because the Food and Drug Administration has determined that the device is not "substantially equivalent" to any device subject to this subpart or under section 520(l) (1) through (3) of the act because the device was regarded previously as a new drug. This subpart does apply to a device that was previously regarded as an antibiotic drug and that is subject to section 520(l)(4) of the act. In classifying a device under this section, the Food and Drug Administration will follow the procedures described in paragraphs (b) through (g) of this section.

(b) The Commissioner refers the device to the appropriate classification panel organized and operated in accordance with section 513 (b) and (c) of the act and part 14 of this chapter.

(c) In order to make recommendations to the Commissioner on the class of regulatory control (class I, class II, or class III) appropriate for the device, the panel reviews the device for safety and effectiveness. In so doing, the panel:

(1) Considers the factors set forth in § 860.7 relating to the determination of safety and effectiveness;

(2) Determines the safety and effectiveness of the device on the basis of the types of scientific evidence set forth in § 860.7;

(3) Answers the questions in the classification questionnaire applicable to the device being classified;

(4) Completes a supplemental data sheet for the device;

(5) Provides, to the maximum extent practicable, an opportunity for interested persons to submit data and views on the classification of the device in accordance with part 14 of this chapter.

(d) Based upon its review of evidence of the safety and effectiveness of the device, and applying the definition of each class in § 860.3(c), the panel submits to the Commissioner a recommendation regarding the classification of the device. The recommendation will include:

(1) A summary of the reasons for the recommendation;

(2) A summary of the data upon which the recommendation is based, accompanied by references to the sources containing such data;

(3) An identification of the risks to health (if any) presented by the device;

(4) In the case of a recommendation for classification into class I, a recommendation as to whether the device should be exempted from the requirements of one or more of the following sections of the act: section 510 (registration, product listing, and premarket notification) section 519 (records and reports) and section 520(f) (good manufacturing practice regulations) in accordance with § 860.95;

(5) In the case of a recommendation for classification into class II or class III, to the extent practicable, a recommendation for the assignment to the device of a priority for the application of a performance standard or a premarket approval requirement;

(6) In the case of a recommendation for classification of an implant or a life-supporting or life-sustaining device into class I or class II, a statement of why premarket approval is not necessary to provide reasonable assurance of the safety and effectiveness of the device, accompanied by references to supporting documentation and data satisfying the requirements of § 860.7, and an identification of the risks to health, if any, presented by the device.

(e) A panel recommendation is regarded as preliminary until the Commissioner has reviewed it, discussed it with the panel if appropriate, and published a proposed regulation classifying the device. Preliminary panel recommendations are filed in the Hearing Clerk's office upon receipt and are available to the public upon request.

(f) The Commissioner publishes the panel's recommendation in the FEDERAL REGISTER, together with a proposed regulation classifying the device, and other devices of that generic type, and provides interested persons an opportunity to submit comments on the recommendation and proposed regulation.

(g) The Commissioner reviews the comments and issues a final regulation classifying the device and other devices of that generic type. The regulation will:

(1) If classifying the device into class I, prescribe which, if any, of the requirements of sections 510, 519, and 520(f) of the act will not apply to the device and state the reasons for making the requirements inapplicable, in accordance with § 860.95;

(2) If classifying the device into class II or class III, at the discretion of the Commissioner, establish priorities for the application to the device of a performance standard or a premarket approval requirement;

(3) If classifying an implant, or life-supporting or life-sustaining device, comply with § 860.93(b).

§ 860.93 Classification of implants, life-supporting or life-sustaining devices.

(a) The classification panel will recommend classification into class III of any implant or life-supporting or life-sustaining device unless the panel determines that such classification is not necessary to provide reasonable assurance of the safety and effectiveness of the device. If the panel recommends classification or reclassification of such a device into a class other than class III, it shall set forth in its recommendation the reasons for so doing together with references to supporting documentation and data satisfying the requirements of § 860.7, and an identification of the risks to health, if any, presented by the device.

(b) The Commissioner will classify an implant or life-supporting or life-sustaining device into class III unless the Commissioner determines that such classification is not necessary to provide reasonable assurance of the safety and effectiveness of the device. If the Commissioner proposes to classify or reclassify such a device into a class other than class III, the regulation or order effecting such classification or reclassification will be accompanied by a full statement of the reasons for so doing. A statement of the reasons for not classifying or retaining the device in class III may be in the form of concurrence with the reasons for the recommendation of the classification panel, together with supporting documentation and data satisfying the requirements of § 860.7 and an identification of the risks to health, if any, presented by the device.

§ 860.95 Exemptions from sections 510, 519, and 520(f) of the act.

(a) A panel recommendation to the Commissioner that a device be classified or reclassified into class I will include a recommendation as to whether the device should be exempted from some or all of the requirements of one or more of the following sections of the act: section 510 (registration, product listing and premarket notification), section 519 (records and reports), and section 520(f) (good manufacturing practice regulations).

(b) A regulation or an order classifying or reclassifying a device into class I will specify which requirements, if any, of sections 510, 519, and 520(f) of the act the device is to be exempted from, together with the reasons for such exemption.

(c) The Commissioner will grant exemptions under this section only if the Commissioner determines that the requirements from which the device is exempted are not necessary to provide reasonable assurance of the safety and effectiveness of the device.

Subpart C—Reclassification

§ 860.120 General.

(a) Sections 513(e) and (f), 514(b), 515(b), and 520(l) of the act provide for reclassification of a device and prescribe the procedures to be followed to effect reclassification. The purposes of subpart C are to:

(1) Set forth the requirements as to form and content of petitions for reclassification;

(2) Describe the circumstances in which each of the five statutory reclassification provisions applies; and

(3) Explain the procedure for reclassification prescribed in the five statutory reclassification provisions.

(b) The criteria for determining the proper class for a device are set forth in § 860.3(c). The reclassification of any device within a generic type of device causes the reclassification of all substantially equivalent devices within that generic type. Accordingly, a petition for the reclassification of a specific device will be considered a petition for reclassification of all substantially equivalent devices within the same generic type.

(c) Any interested person may submit a petition for reclassification under section 513(e), 514(b), or 515(b). A manufacturer or importer may submit a petition for reclassification under section 513(f) or 520(l).

§ 860.123 Reclassification petition: content and form.

(a) Unless otherwise provided in writing by the Commissioner, any petition for reclassification of a device, regardless of the section of the act under which it is filed, shall include the following:

(1) A specification of the type of device for which reclassification is requested;

(2) A statement of the action requested by the petitioner, e.g., "It is requested that — device(s) be reclassified from class III to a class II";

(3) A completed supplemental data sheet applicable to the device for which reclassification is requested;

(4) A completed classification questionnaire applicable to the device for which reclassification is requested;

(5) A statement of the basis for disagreement with the present classification status of the device;

(6) A full statement of the reasons, together with supporting data satisfying the requirements of § 860.7, why the device should not be classified into its present classification and how the proposed classification will provide reasonable assurance of the safety and effectiveness of the device;

(7) Representative data and information known by the petitioner that are unfavorable to the petitioner's position;

(8) If the petition is based upon new information under section 513(e), 514(b), or 515(b) of the act, a summary of the new information;

(9) Copies of source documents from which new information used to support the petition has been obtained (attached as appendices to the petition).

(b) Each petition submitted pursuant to this section shall be:

(1) Addressed to the Food and Drug Administration, Bureau of Medical Devices, Document Control Center (HFK-20), 8757 Georgia Avenue, Silver Spring, Md. 20910;

(2) Marked clearly with the section of the act under which the petition is being submitted, i.e., "513(e)," "513(f)," "514(b)," "515(b)," or "520(l) Petition";

(3) Bound in a volume or volumes, where necessary; and

(4) Submitted in quintuplicate.

§ 860.125 Consultation with panels.

(a) When the Commissioner is required to refer a reclassification petition to a classification panel for its recommendation under § 860.134, or is required, or chooses, to consult with a panel concerning a reclassification petition, such as under § 860.130, § 860.132, or § 860.136, the Commissioner will distribute a copy of the petition, or its relevant portions, to each panel member and will consult with the panel in one of the following ways:

(1) Consultation by telephone with at least a majority of current voting panel members and, when possible, nonvoting panel members;

(2) Consultation by mail with at least a majority of current voting panel members and, when possible, nonvoting panel members; and

(3) Discussion at a panel meeting.

(b) The method of consultation chosen by the Commissioner will depend upon the importance and complexity of the subject matter involved and the time available for action. When time and circumstances permit, the Commissioner will consult with a panel through discussion at a panel meeting.

(c) When a petition is submitted under § 860.134 for a post-enactment, not substantially equivalent device ("new device"), in consulting with the panel the Commissioner will obtain a recommendation that includes the information described in § 860.84(d). In consulting with a panel about a petition submitted under § 860.130, § 860.132, or § 860.136, the Commissioner may or may not obtain a formal recommendation.

§ 860.130 General procedures under section 513(e) of the act.

(a) Section 513(e) of the act applies to reclassification proceedings under the act based upon new information.

(b) A proceeding to reclassify a device under section 513(e) may be initiated:

(1) On the initiative of the Commissioner alone;

(2) On the initiative of the Commissioner in response to a request for change in classification based upon new information, under section 514(b) or 515(b) of the act (see § 860.132); or

(3) In response to the petition of an interested person, based upon new information, filed in accordance with § 860.123.

(c) The rulemaking procedures in § 10.40 of this chapter apply to proceedings to reclassify a device under section 513(e), except that the Commissioner may secure a recommendation with respect to a proposed reclassification from the classification panel to which the device was last referred. The panel will consider a proposed reclassification submitted to it by the Commissioner in accordance with the consultation procedures of § 860.125. Any recommendation submitted to the Commissioner by the panel will be published in the FEDERAL REGISTER when the Commissioner promulgates a regulation under this section.

(d) Within 180 days after the filing of a petition for reclassification under this section, the Commissioner, by order published in the FEDERAL REGISTER, will either deny the petition or give notice of his intent to initiate a change in the classification of the device.

(e) If a device is reclassified under this section, the regulation effecting the reclassification may revoke any performance standard or premarket approval requirement that previously applied to the device but that is no longer applicable because of the change in classification.

(f) A regulation under this section changing the classification of a device from class III to class II may provide that such classification will not take effect until the effective date of a performance standard for the device established under section 514 of the act.

§ 860.132 Procedures when the Commissioner initiates a performance standard or premarket approval proceeding under section 514(b) or 515(b) of the act.

(a) Sections 514(b) and 515(b) of the act require the Commissioner to provide, by notice in the FEDERAL REGISTER, an opportunity for interested parties to request a change in the classification of a device based upon new information relevant to its classification when the Commissioner initiates a proceeding either to develop a performance standard for the device if in class II, or to promulgate a regulation requiring premarket approval for the device if in class III. In either case, if the Commissioner agrees that the new information warrants a change in classification, the Commissioner will publish in the FEDERAL REGISTER notice of the Commissioner's intent to initiate a proceeding under section 513(e) of the act and § 860.130 to effect such a change.

(b) The procedures for effecting a change in classification under sections 514(b) and 515(b) of the act are as follows:

(1) Within 15 days after publication of the Commissioner's notice referred to in paragraph (a) of this section, an interested person files a petition for reclassification in accordance with § 860.123.

(2) The Commissioner consults with the appropriate classification panel with regard to the petition in accordance with § 860.125.

(3) Within 60 days after publication of the notice referred to in paragraph (a) of this section, the Commissioner, by order published in the FEDERAL REGISTER, either denies the petition or gives notice of his intent to initiate a change in classification in accordance with § 860.130.

§ 860.134 Procedures for "new devices" under section 513(f) of the act.

(a) Section 513(f)(2) of the act applies to reclassification proceedings initiated by a manufacturer or importer for reclassification of a device currently in class III by operation of section 513(f)(1) of the act. This category includes any device that is to be first introduced or delivered for introduction into interstate commerce for commercial distribution after May 28, 1976, unless:

(1) It is substantially equivalent to another device that was in commercial distribution before that date and had not been regulated before that date as a new drug; or

(2) It is substantially equivalent to another device that was not in commercial distribution before such date but which has been classified into class I or class II; or

(3) The Commissioner has classified the device into class I or class II in response to a petition for reclassification under this section.

The Commissioner determines whether a device is "substantially equivalent" for purposes of the application of this section. If a manufacturer or importer believes that a device is not "substantially equivalent" but that it should not be in class III under the criteria in § 860.3(c), the manufacturer or importer may petition for reclassification under this section. A

manufacturer or importer who believes that a device is "substantially equivalent" and wishes to proceed to market the device shall submit a premarket notification in accordance with part 807 of this chapter. After considering a premarket notification, the Commissioner will determine whether the device is "substantially equivalent" and will notify the manufacturer or importer of such determination in accordance with part 807 of this chapter.

(b) The procedures for effecting reclassification under section 513(f) of the act are as follows:

(1) The manufacturer or importer of the device petitions for reclassification of the device in accordance with § 860.123.

(2) Within 30 days after the petition is filed, the Commissioner notifies the petitioner of any deficiencies in the petition that prevent the Commissioner from making a decision on it and allows the petitioner to supplement a deficient petition. Within 30 days after any supplemental material is received, the Commissioner notifies the petitioner whether the petition, as supplemented, is adequate for review.

(3) After determining that the petition contains no deficiencies precluding a decision on it, the Commissioner refers the petition to the appropriate classification panel for its review and recommendation whether to approve or deny the petition.

(4) Within 90 days after the date the petition is referred to the panel, following the review procedures set forth in § 860.84(c) for the original classification of an "old" device, the panel submits to the Commissioner its recommendation containing the information set forth in § 860.84(d). A panel recommendation is regarded as preliminary until the Commissioner has reviewed it, discussed it with the panel, if appropriate, and developed a proposed reclassification order. Preliminary panel recommendations are filed in the hearing clerk's office upon receipt and are available to the public upon request.

(5) The panel recommendation is published in the FEDERAL REGISTER as soon as practicable and interested persons are provided an opportunity to comment on the recommendation.

(6) Within 90 days after the panel's recommendation is received (and no more than 210 days after the date the petition was filed), the Commissioner denies or approves the petition by order in the form of a letter to the petitioner. If the Commissioner approves the petition, the order will classify the device into class I or class II in accordance with the criteria set forth in § 860.3(c) and subject to the applicable requirements of § 860.93, relating to the classification of implants, life-supporting or life-sustaining devices, and § 860.95, relating to exemptions from certain requirements of the act.

(7) Within a reasonable time after issuance of an order under this section, the Commissioner announces the order by notice published in the FEDERAL REGISTER.

§ 860.136 Procedures for transitional products under section 520(l) of the act.

(a) Section 520(l)(2) of the act applies to reclassification proceedings initiated by a manufacturer or importer for reclassification of a device currently in class III by operation of section 520(l)(1) of the act. This section applies only to devices that the Food and Drug Administration regarded as "new drugs" before May 28, 1976.

(b) The procedures for effecting reclassification under section 520(l) are as follows:

(1) The manufacturer or importer of the device files a petition for reclassification of the device in accordance with § 860.123.

(2) Within 30 days after the petition is filed, the Commissioner notifies the petitioner of any deficiencies in the petition that prevent the Commissioner from making a decision on it, allowing the petitioner to supplement a deficient petition. Within 30 days after any supplemental material is received, the Commissioner notifies the petitioner whether the petition, as supplemented, is adequate for review.

(3) The Commissioner provides the petitioner an opportunity for a regulatory hearing conducted in accordance with part 16 of this chapter.

(4) The Commissioner consults with the appropriate classification panel with regard to the petition in accordance with § 860.125.

(5) Within 180 days after the petition is filed (where the Commissioner has determined it to be adequate for review), the Commissioner, by order in the form of a letter to the petitioner, either denies the petition or classifies the device into class I or class II in accordance with the criteria set forth in § 860.3(c).

(6) Within a reasonable time after issuance of an order under this section, the Commissioner announces the order by notice published in the FEDERAL REGISTER.

Effective date: This regulation shall be effective August 28, 1978.

(Secs. 513, 514, 515, 519, 520, 701(a), 52 Stat. 1055, 90 Stat. 540-559, 564-574 (21 U.S.C. 360c, 360d, 360e, 360i, 360j, and 371(a)).)

Dated: July 18, 1978.

SHERWIN GARDNER,
Acting Commissioner of Food and Drugs.

[FR Doc. 78-20625 Filed 7-27-78; 8:45 am]

federal register

FRIDAY, JULY 28, 1978
PART III

DEPARTMENT OF LABOR

Employment Standards Administration

■

MINIMUM WAGES FOR FEDERAL AND FEDERALLY ASSISTED CONSTRUCTION

General Wage Determination Decisions

[4510-27]

DEPARTMENT OF LABOR

Employment Standards Administration

MINIMUM WAGES FOR FEDERAL AND FEDERALLY ASSISTED CONSTRUCTION

General Wage Determination Decisions

General wage determination decisions of the Secretary of Labor specify, in accordance with applicable law and on the basis of information available to the Department of Labor from its study of local wage conditions and from other sources, the basic hourly wage rates and fringe benefit payments which are determined to be prevailing for the described classes of laborers and mechanics employed in construction activity of the character and in the localities specified therein.

The determinations in these decisions of such prevailing rates and fringe benefits have been made by authority of the Secretary of Labor pursuant to the provisions of the Davis-Bacon Act of March 3, 1931, as amended (46 Stat. 1494, as amended, 40 U.S.C. 276a) and of other Federal statutes referred to in 29 CFR 1.1 (including the statutes listed at 36 FR 306 following Secretary of Labor's order No. 24-70) containing provisions for the payment of wages which are dependent upon determination by the Secretary of Labor under the Davis-Bacon Act; and pursuant to the provisions of part 1 of subtitle A of title 29 of Code of Federal Regulations, procedure for predetermination of wage rates (37 FR 21138), and of Secretary of Labor's orders 12-71 and 15-71 (36 FR 8755, 8756). The prevailing rates and fringe benefits determined in these decisions shall, in accordance with the provisions of the foregoing statutes, constitute the minimum wages payable on Federal and federally assisted construction projects to laborers and mechanics of the specified classes engaged on contract work of the character and in the localities described therein.

Good cause is hereby found for not utilizing notice and public procedure thereon prior to the issuance of these determinations as prescribed in 5 U.S.C. 553 and not providing for delay in effective date as prescribed in that section, because the necessity to issue construction industry wage determination frequently and in large volume causes procedures to be impractical and contrary to the public interest.

General wage determination decisions are effective from their date of publication in the FEDERAL REGISTER without limitation as to time and are to be used in accordance with the provisions of 29 CFR parts 1 and 5. Accordingly, the applicable decision together with any modification issued subsequent to its publication date shall be made a part of every contract for performance of the described work within the geographic area indicated as required by an applicable Federal prevailing wage law and 29 CFR part 5. The wage rates contained therein shall be the minimum paid under such contract by contractors and subcontractors on the work.

MODIFICATIONS AND SUPERSEDEAS DECISIONS TO GENERAL WAGE DETERMINATION DECISIONS

Modifications and supersedeas decisions to general wage determination decisions are based upon information obtained concerning changes in prevailing hourly wage rates and fringe benefit payments since the decisions were issued.

The determinations of prevailing rates and fringe benefits made in the modifications and supersedeas decisions have been made by authority of the Secretary of Labor pursuant to the provisions of the Davis-Bacon Act of March 3, 1931, as amended (46 Stat. 1494, as amended, 40 U.S.C. 276a) and of other Federal statutes referred to in 29 CFR 1.1 (including the statutes listed at 36 FR 306 following Secretary of Labor's order No. 24-70) containing provisions for the payment of wages which are dependent upon determination by the Secretary of Labor under the Davis-Bacon Act; and pursuant to the provisions of part 1 of subtitle A of title 29 of Code of Federal Regulations, procedure for predetermination of wage rates (37 FR 21138) and of Secretary of Labor's orders 13-71 and 15-71 (36 FR 8755, 8756). The prevailing rates and fringe benefits determined in foregoing general wage determination decisions, as hereby modified, and/or superseded shall, in accordance with the provisions of the foregoing statutes, constitute the minimum wages payable on Federal and federally assisted construction projects to laborers and mechanics of the specified classes engaged in contract work of the character and in the localities described therein.

Modifications and supersedeas decisions are effective from their date of publication in the FEDERAL REGISTER without limitation as to time and are to be used in accordance with the provisions of 29 CFR parts 1 and 5.

Any person, organization, or governmental agency having an interest in the wages determined as prevailing is encouraged to submit wage rate information for consideration by the Department. Further information and self-explanatory forms for the purpose of submitting this data may be obtained by writing to the U.S. Department of Labor, Employment Standards Administration, Office of Special Wage Standards, Division of Wage Determinations, Washington, D.C. 20210. The cause for not utilizing the rulemaking procedures prescribed in 5 U.S.C. 553 has been set forth in the original general wage determination decision.

NEW GENERAL WAGE DETERMINATION DECISIONS

Indiana—IN78-2066.

MODIFICATIONS TO GENERAL WAGE DETERMINATION DECISIONS

The numbers of the decisions being modified and their dates of publication in the FEDERAL REGISTER are listed with each State.

California:	
CA78-5107	July 7, 1978.
District of Columbia:	
DC78-3008	Mar. 17, 1978.
Florida:	
FL78-1062	July 14, 1978.
Iowa:	
IA77-4223; IA77-4224; IA77-4225; IA77-4226; IA77-4227; IA77-4228; IA77-4229; IA77-4230; IA77-4231; IA77-4232; IA77-4233; IA77-4234; IA77-4235	Sept. 30, 1977.
North Carolina:	
NC78-1061	July 7, 1978.
Oklahoma:	
OK77-4060	Mar. 11, 1977.
Pennsylvania:	
PA77-3122	Sept. 9, 1977.
PA78-3037	Apr. 21, 1978.
Tennessee:	
TN78-1058	July 7, 1978.

SUPERSEDEAS DECISIONS TO GENERAL WAGE DETERMINATION DECISIONS

The numbers of the decisions being superseded and their dates of publication in the FEDERAL REGISTER are listed with each State. Supersedeas decision numbers are in parentheses following the numbers of the decisions being superseded.

Arizona:	
AZ77-5115 (AZ78-5025); AZ77-5026 (AZ78-5116)	June 17, 1977.
Arkansas:	
AR78-4070 (AR78-4074)	June 30, 1978.
Connecticut:	
CT78-3003 (CT78-3055); CT78-3004 (CT78-3056)	Feb. 17, 1978.
Louisiana:	
AR78-4070 (AR78-4074)	June 30, 1978.
Mississippi:	
AR78-4070 (AR78-4074)	Do.
Tennessee:	
AR78-4070 (AR78-4074)	Do.
Vermont:	
VT76-2170 (VT78-2067)	Dec. 10, 1976.

CANCELLATION OF GENERAL WAGE DETERMINATION DECISIONS

None.

Signed at Washington, D.C., this 21st day of July 1978.

XAVIER M. VELA,
Adminstrator,
Wage and Hour Division.

NEW DECISION

STATE: INDIANA
DECISION NO.: IN78-2066
DESCRIPTION OF WORK: Building Construction (Does not include single family homes & garden type apartments up to & including 4 stories)

COUNTIES: *See below
DATE: Date of Publication

*Elkhart, Jasper, Kosciusko, LaGrange, Marshall, Newton, Pulaski, & Starke

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
ASBESTOS WORKERS:					
Elkhart, Jasper, Kosciusko, Marshall, Pulaski, & Starke Cos.	$12.13	.65	.90		
LaGrange Co.	13.15	.50	.75		
Newton Co.	12.11	.81	.72		.12
BOILERMAKERS	12.55	.80	1.00		.03
BRICKLAYERS; Stonemasons					
Elkhart, Kosciusko, & LaGrange Cos.	11.43		.30		.02
Jasper, Newton & Starke Cos.	11.85	.75	.55		.05
Marshall Co.	11.36	1.00	.90		.06
Pulaski Co.	10.10	1.00	.40		
CARPENTERS; Millwrights; Piledrivermen; & Soft Floor Layers:					
Elkhart Co.					
Carpenters; Soft Floor Layers	10.65	.70	.55		.08
Millwrights; Piledrivermen	10.90	.70	.55		.08
Jasper, Newton, & Starke Cos.:					
Carpenters; Soft Floor Layers	12.78	.65	.67		.05
Millwrights	12.88	.65	.67		.05
Kosciusko (Warsaw & Vic), Marshall, & Pulaski Cos.:					
Carpenters; Soft Floor Layers	10.23	.80	.60		.05
Millwrights	10.73	.80	.60		.05
Piledrivermen	10.43	.80	.60		.05
Kosciusko (Remaining Portion) Co.	10.90	.60	.85		.08
LaGrange Co.:					
Carpenters; Soft Floor Layers	10.57	6%a			.08
Millwrights; Piledrivermen	10.90	6%a			.08
CEMENT MASONS:					
Elkhart, Kosciusko, & LaGrange Cos.	10.48		.30		.02
Jasper (Northern 1/3), Pulaski (Northern 2/3), & Starke Cos.	10.95	.90	1.00		.06
Jasper (Remaining Portion), & Newton (Southeastern 2/3) Cos.	10.00	.60	.75		.03
Newton (Northern 1/3)	11.42	.90	.60		
ELECTRICIANS:					
Elkhart, Kosciusko, & Marshall Cos.	11.85	4.8%	7%		.2%
Jasper, Pulaski, & Starke Cos.	12.10	4%	7½%		.5%
LaGrange Co.	11.60	.50	3%+.30		.06
Newton Co.	12.60	6.5%	8.3%		.7%

DECISION NO. IN78-2066

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
ELEVATOR CONSTRUCTORS:					
Elkhart, Jasper, Kosciusko (NW Portion), LaGrange (NW of city of LaGrange), Marshall, Pulaski, & Starke Cos.					
Elevator Constructors	$11.115	.895	.69	b&c	.035
Helpers	70%JR	.895	.69	b&c	.035
Helpers (Prob).	50%JR				
Kosciusko (SE part), & LaGrange (Remaining Portion) Cos.:					
Elevator Constructors	11.195	.895	.69	b&c	.035
Helpers	70%JR	.895	.69	b&c	.035
Helpers (Prob.)	50%JR				
Newton Co.:					
Elevator Constructors	12.740	.895	.69	b&c	.035
Helpers	70%JR	.895	.69	b&c	.035
Helpers (Prob.)	50%JR				
GLAZIERS:					
Kosciusko (Southern portion), & LaGrange Cos.	10.20		.25	.40d	.04
IRONWORKERS:					
Elkhart, Kosciusko (NW portion exclu. Warsaw), LaGrange (W ½ exclu. city of LaGrange), Marshall, Pulaski, & Starke Cos.	11.45	.90	1.33		.03
Jasper (N½), & Newton Cos.	12.72	.80	1.66		.09
Jasper (S½) Co.	11.14	.90	1.25		.02
Kosciusko (SW portion inclu. Warsaw), & LaGrange (E½ of Co. inclu. city of LaGrange) Cos.	11.80	.90	1.45		.02
LATHERS:					
Elkhart, Kosciusko, LaGrange, Marshall & Starke Cos.	10.12	.43	.50		.01
Jasper (So. of Hwy 14) Co.	10.54	.55	.35		.01
MARBLE SETTERS; Terrazzo Workers; & Tile Setters:					
Elkhart, Kosciusko, & LaGrange Cos.:					
Terrazzo Workers; & Tile Setters	10.20		.30	e	.02
Jasper, Newton, & Starke Cos.:					
Marble Setters	11.85	.75	.55		.05
Tile Setters	11.75	.75	.55		.02
Marshall, & Pulaski Cos.	9.65	1.00	.90	f	.01

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	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
PAINTERS:					
Elkhart, Kosciusko, Marshall, Pulaski, & Starke Cos.:					
Brush; Drywall tapere & Finishers	9.59		.40		$15.00p/yr
Jasper & Newton Cos.:					
Brush	10.50	.64	.50		.05
Paperhanging	10.75	.64	.50		.05
Sandblasting; Spray	11.25	.64	.50		.05
Drywall taping	11.55	.64	.50		.05
LaGrange Co.:					
Brush; Paperhangers; Rollers; & Tapers	8.60	.42	.45		.10
Sandblasting; Spray; & Steam cleaning	9.60	.42	.45		.10
PIPEFITTERS; Plumbers; & Steamfitters:					
Elkhart, Kosciusko, & LaGrange Cos.	12.15	.55	.85		.07
Jasper (N. side of city of Rensselaer, S.), Marshall, Pulaski, & Starke Cos.	11.25	.48	.95		.10
Pipefitters; Steamfitters:					
Jasper (Rem. of Co.), & Newton Cos.	12.90	.80	1.00		.02
Plumbers:					
Jasper (Rem. of Co.) Co.	12.20	.90	1.50		.04
Newton Co.	12.22	1.00	1.15		.04
PLASTERERS:					
Elkhart, Kosciusko, & LaGrange Cos.	10.28		.30		.02
Jasper (NE portion of Co. w. to, but not inclu. Wheatfield), Pulaski (N 2/3 of Co.), & Starke Cos.	10.87	.90	1.00		.06
Newton Co. (N½ of Co.)	10.21	.90	.60		.01
ROOFERS:					
Elkhart, Kosciusko, Marshall, Pulaski, & Starke Cos.:					
Composition, Damp & Waterproof	10.95	.50	.40		.03
Slate, Tile, & Asbestos	11.45	.50	.40		.03
Jasper & Newton Cos.	12.21	.70	.50		
LaGrange Co.:					
Roofers	10.90		.10		
Pitch	11.15		.10		

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	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
SHEET METAL WORKERS:					
Elkhart, Kosciusko, & Marshall Cos.	$10.98	.65	.615		.035
Jasper, Newton, Pulaski, & Starke Cos.	12.15	.75	1.16		.12
LaGrange Co.	11.98	.50	.60		.12
SPRINKLER FITTERS	12.10	.75	1.05		.08

PAID HOLIDAYS:
A-New Year's Day; B-Memorial Day; C-Independence Day; D-Labor Day; E-Thanksgiving Day; F-Christmas Day

FOOTNOTES:
a. 6% for health and welfare includes pension.
b. 7 paid holidays: A through F, and Day after Thanksgiving Day.
c. Employer contributes 8% of regular hourly rate to vacation pay credit for employee who has worked in business more than 5 years; 6% for employee who has worked in business less than 5 years.
d. 6 paid holidays: A through F provided such employees work the last scheduled day prior to and the next scheduled work day after the holiday unless permission for not working on such days is granted by the employer.
e. 6 paid holidays: A through F
f. 6 paid holidays: A through F

TRUCK DRIVERS Elkhart, Kosciusko, LaGrange, & Marshall Counties	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
PICK-UPS	$6.94	$13.50a	$ 22.00a	b	
SINGLE AXLES	7.15	13.50a	22.00a	b	
TANDEMS; Fuels	7.26	13.50a	22.00a	b	
TRI AXLES	7.31	13.50a	22.00a	b	
SEMI-TRAILERS	7.36	13.50a	22.00a	b	

FOOTNOTES:
a. Per week per employee
b. 1 week's paid vacation for 3 years' service, 2 weeks' paid vacation for 10 years' service & 3 weeks' paid vacation for 15 years' service.

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LABORERS	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
Elkhart, Kosciusko, LaGrange, & Marshall Counties					
GROUP I	$8.25	.70	.50		.09
GROUP II	8.45	.70	.50		.09
GROUP III	8.55	.70	.50		.09
GROUP IV	9.25	.70	.50		.09
Jasper & Newton Counties					
GROUP I	8.70	.70	.55		.09
GROUP II	8.90	.70	.55		.09
GROUP III	9.00	.70	.55		.09
GROUP IV	9.70	.70	.55		.09
Pulaski County					
GROUP I	7.70	.70	.50		.09
GROUP II	7.90	.70	.50		.09
GROUP III	8.00	.70	.50		.09
GROUP IV	8.70	.70	.50		.09
Starke County					
GROUP I	8.50	.70	.50		.09
GROUP II	8.70	.70	.50		.09
GROUP III	8.80	.70	.50		.09
GROUP IV	9.50	.70	.50		.09

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LABORERS

GROUP I: Building & Construction laborers; Scaffold builders (other than for masons or plasterers); Ironworker helpers; Mechanic tenders; Civil engineer helpers & surveyor helpers; Rodmen & Chainmen; Window washers & cleaners; Waterboys & Toolhousemen; Roofer's helpers; Railroad workers; Masonry wall washers (interior & exterior); Cement finisher helpers; Carpenter helpers; Helpers of all other crafts not listed; Mason tenders for Areas I, IA, IB, and Counties of Adams, Allen, DeKalb, Steuben, Huntington, Noble, Wabash, Wells, & Whitley; All portable water pumps with discharge up to 3 inches

GROUP II: Waterproofing; Handling of creosote lumber or like treated material (excluding railroad material); Asphalt rakers & lutmen; Kettlemen; Air tool operators, vibrators, chipping hammer operators and all pneumatic tool operators; Earth compactors; Jackmen & sheetmen working ditches deeper than 6 ft. in depth; Laborers working ditches 6 ft. in depth or deeper; Assembly of Unicrete pump; Chain saw operators; Tile layers (sewer or field) & sewer pipe layers (metallic or non-metallic); Motor driven wheelbarrows & concrete buggies; Hyster operators; Pump crete assemblers; Conveyor assemblers; Core drill operators; Cement, lime or silica Clay handlers (bulk or bag); Handling of toxic materials damaging to clothing; Pneumatic spikers; Deck engine & winch operators; Water main & cable ducking (metallic & non-metallic)

GROUP III: Plasterers' tenders; Mason tenders, except for Areas I, IA, IB, and Counties of Adams, Allen, DeKalb, Steuben, Huntington, Noble, Wabash, Wells, & Whitley; Mortar mixers; Welders (acetylene or electric); Cutting torch or burner; Cement nozzle laborers; Cement gun operators; Scaffold builders when working for plasterers; Scaffold builders when working for masons (except in Areas I, IA, IB, and Counties of Adams, Allen, DeKalb, Steuben, Huntington, Noble, Wabash, Wells, & Whitley)

GROUP IV: Dynamite men

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POWER EQUIPMENT OPERATORS

Elkhart, Kosciusko, LaGrange, & Marshall Counties	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vocation	Education and/or Appr. Tr.
GROUP I	$10.52	.65	.85	.40	.05
GROUP II	9.16	.65	.85	.40	.05
GROUP III	8.38	.65	.85	.40	.05
GROUP IV	7.70	.65	.85	.40	.05

CLASSIFICATIONS

GROUP I: All power cranes; Truck cranes; Locomotive cranes; LeTourneau Tournapulls; Shovels; Draglines; Derricks; Pile drivers; Highlifts; Tractors w/Side boom or "A" Frame; Forklifts 18' 6" in height or over; Motor patrols; Combination backhoe & loader; Mechanic; Conveyor systems; Hoist (3 drum) & over

GROUP II: All bulldozers; Scoops; Push cats; Concrete mixers of more than 21 cu. ft. capacity; Locomotives; Rollers; Stone crushers; Fork lifts under 18' 6"; Air compressors - 600 cu. ft. & over; Combination of Gasoline or Deisel driven welding machine & compressor; Engineers operating throttle valve with boiler and compressor for piledriving; Concrete pumps; Trench machines excluding ditch witches; Cableways; Hoist (2 drum)

GROUP III: Truck winches w/A Frame & Power winches; Tractors-farm type; Drills & conveyors; Small rubber tire end loaders ½ cu. yd. & under; Bobcat

GROUP IV : Gin poles; Sasgen derricks & similar hoists; Firemen; Oilers; One drum hoist; Single drum tugger hoist; Automatic hoist; Elevators; Compressor over 210 cu. ft. & under 600 cu. ft.; Well points

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POWER EQUIPMENT OPERATORS

Jasper, Newton, Pulaski, & Starke Counties	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vocation	App. Tr.
Class I	$ 10.30	.65	.85		.05
Class II	9.80	.65	.85		.05
Class III	8.25	.65	.85		.05
Class IV	7.25	.65	.85		.05

CLASS I Mechanic; asphalt plant; autograde; batch plant; benoto (requires two engineers); boiler & throttle valve; caisson rigs; central redimix plant; combination backhoe, front end loader with backhoe bucket of 1 cu. yd. & over; combination tugger hoist & air compressor; compressor and throttle; concrete breaker (truck mounted); concrete conveyor; concrete paver over 27E cu. ft.; concrete paver 27E cu. ft. and under; concrete tower; cranes, all; cranes, tower; derricks, all; derricks, traveling; fork-lift-lull type; forklift - 10 ton and over; hoists, one, two, and three drum; hoists, two tugger one floor; hydraulic boom truck; locomotive, all; pile drivers and skid rig; pit machines; pre-stress machine; pump cretes and similar types; rock drill (self-propelled); rock drill (truck mounted); slipform paver; straddle buggies; tractor with boom & side boom; trenching machine; winch tractors

CLASS II Asphalt spreader; boiler; bulldozers; combination backhoe, front-end loader with backhoe bucket, less than 1 cu. yd.; engineer acting as conductor in charge of crew; grader, elevating; greaser engineer; grouting machines; highlift shovels or front endloader; hoists, automatic; cowboy drilling machines; hoists all elevators; hoists, tugger single drum; motor patrol; post hole digger; rollers, all; scoops - tractor drawn; stone crushers; tournapull; winch trucks

CLASS III Concrete mixer (2 bag & over); conveyor, portable; steam generators; tractors, farm & similar type; air compressor small 150 & under (1 to 5 not to exceed a total of 300 ft.); air compressor - large over 150 combination - small equipment operator; forklift trucks; generators; pumps (1 to 3 not to exceed a total of 300 ft.); pumps, well points; welding machines (2 through 5); winches ¼ electric drill winches

CLASS IV Heaters, mechanical (1 to 5); oilers & switchmen

DECISION NO. IN78-2066

Jasper & Newton Counties	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
TRUCK DRIVERS:					
Pickup trucks	$7.64	$19.00a	$31.00a	b & c	
Helpers; Greasers; Tiremen	7.74	19.00a	31.00a	b & c	
Single axles; Straight trucks; & Warehousemen	7.79	19.00a	31.00a	b & c	
Tandem axle & Dogleg straight trucks	7.89	19.00a	31.00a	b & c	
Bituminous Distributors	7.94	19.00a	31.00a	b & c	
Mechanics; Tri-axles; Semi trucks	8.09	19.00a	31.00a	b & c	

FOOTNOTES:

a. Per week per employee

b. Six paid holidays: New Year's Day; Memorial Day; Independence Day; Labor Day; Thanksgiving Day; & Christmas Day

c. One week's paid vacation after one year's employment; Two weeks' paid vacation after three years' employment; Three weeks' paid vacation after ten years' employment.

DECISION NO. CA78-5107 - Mod. #1 (43 FR 29444 - July 7, 1978)	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
Alameda, Alpine, Amador, Butte, Calaveras, Colusa, Contra Costa, Del Norte, Eldorado, Fresno, Glenn, Humboldt, Kings, Lake, Lassen, Madera, Marin, Mariposa, Mendocino, Merced, Modoc, Monterey, Napa, Nevada, Placer, Plumas, Sacramento, San Benito, San Francisco, San Joaquin, San Mateo, Santa Clara, Santa Cruz, Shasta, Sierra, Siskiyou, Solano, Sonoma, Stanislaus, Sutter, Tehama, Trinity, Tulare, Tuolumne, Yolo and Yuba Counties, California					
Add:					
Painters:					
Sacramento, Sierra, Solano Counties (excluding portions of Counties in the Lake Tahoe Area)					
Brush	$12.31	1.10	1.50	.80	.06
Spray	12.81	1.10	1.50	.80	.06
Tapers	13.11	1.10	1.50	.80	.06
Change:					
Decision reference numbers on pages 29461, 29462 and 29463 to Decision No. CA78-5107					

DECISION #DC78-3008 - Mod. # 5 (43 FR 11464 - March 17, 1978) District of Columbia; Maryland-Montgomery and Prince Georges; and D. C. Training School; - Virginia - Independent City of Alexandria & Arlington	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
Change:					
WATER & SEWER LINES: (District of Columbia and Montgomery County Maryland)					
Power Equipment Operators:					
Backhoes, cable ways, canes, draglines power shovels, tunnel shovels, tunnel mucking machines, derricks, 1 cu. yd. and over	$8.49	.35	.45		.05
Backhoes, cableways, cranes derricks, dragline, tunnel shovels, tunnel mucking machines up to 1 cu. yd., boom cats, elevating graders, hoists, paving mixers, pile-driving engines, batch plants concrete pumps	8.24	.35	.45		.05
Trenching machines (above 8' 3")	8.04	.35	.45		.05
Backhoes (hydraulic, under ½ c. y.)	8.12	.35	.45		.05
Trenching machines (up to 8' 3"), boilers skelton, well drilling machines	9.94	.35	.45		.05
Air compressors, tunnel	7.91	.35	.45		.05
Front end loaders (high lift), bulldozers	7.89	.35	.45		.05
Concrete mixers, power wheel scoops and scrapers, motor graders, tunnel motor men, blade graders, tunnel mechanics	7.79	.35	.45		.05
Mechanics	7.77	.35	.45		.05
Bulldozer, hydraulic tampers	7.69	.35	.45		.05
Roller	7.59	.35	.45		.05
Air compressors, pump, welding machine well points	7.515	.35	.45		.05

DECISION #DC78-3008 - Mod. # 5, Cont'd.	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
Add:					
Building Schedule					
Motor Repairman (Removal and reinstalling of electrical motors)	$9.87	.70	3%+.80	h	

Footnote:

h. Employer contributes to employee who have worked six (6) months or more, shall be given two and one-half days vacation or equivalent thereof. All men in the employ of the employer for one (1) year or more shall be given one week's vacation with pay or the equivalent thereof. All men in the employ of the employer three (3) years or more shall be given two (2) week's vacation with pay or the equivalent thereor. All men in the employ of the employer ten (10) years or more shall be given three (3) weeks vacation with pay or the equivalent thereof.

Omit:					
Laborers:					
Residential over 4 stories:					
Unskilled	5.09	.35			.05
Scaffold builders, paving breakers and mortar mixers	5.24	.35			.05
Work below 6 feet depth	5.34	.35			.05

DECISION #FL78-1062 - Mod. #1 (43 FR 30456 - July 14, 1978) Pinellas County, Florida					
Change:					
Carpenters	$6.56				
Electricians	6.60				
Sheet metal workers	8.00	.85	.55		.06

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
Decision #IA77-4223-Mod. #4					
(42 FR-53002-September 30, 1977)					
Black Hawk County, Iowa					
Change:					
Boilermakers	12.30	.85	1.00		.02
Carpenters	9.09	.45	1.25		
Cement Masons	9.05	.45	1.00		
Electricians	12.22	.45	3%		3/4%
Cable Splicers	12.59	.45	3%		3/4%
Ironworkers	12.18				.06
Line Construction:					
Group 1	10.12	.45	7%	a	½%
Group 2	6.58	.45	7%	a	½%
Group 3	6.78	.45	7%	a	½%
Group 4	8.10	.45	7%	a	½%
Group 5	5.57	.45	7%	a	½%
Millwrights	9.59	.45	1.25		
Piledrivermen	9.09	.45	1.25		
Truck Driver	7.95	.45	.75		
Helpers	7.78	.45	.75		
Decision #IA77-4224-Mod. #5					
(42 FR-53004-September 30, 1977)					
Cerro Gordo County, Iowa					
Change:					
Boilermakers	12.30	.85	1.00		.02
Bricklayers	10.64		.75		
Electricians	12.19	.45	3%		3/4%
Line Construction:					
Group 1	10.12	.45	7%	a	½%
Group 2	6.58	.45	7%	a	½%
Group 3	6.78	.45	7%	a	½%
Group 4	8.10	.45	7%	a	½%
Group 5	5.57	.45	7%	a	½%

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
Decision #IA77-4225-Mod. #3					
(42 FR-53006-September 30, 1977)					
Clinton County, Iowa					
Change:					
Boilermakers	12.30	.85	1.00		.02
Carpenters	10.30	.45	.70		.04
Glaziers	10.59	.70	1.00		.02
Painters					
Roller, steel	10.27	.55	.85		.18
Spray, structural steel	10.77	.55	.85		.18
Piledrivermen	10.70	.45	.70		.04
Decision #IA77-4228-Mod. #4					
(42 FR-53011-September 30, 1977)					
Johnson County, Iowa					
Change:					
Boilermakers	12.30	.85	1.00		.02
Cement Masons	10.78	.45			
Glaziers	10.59	.70	1.00		.02
Ironworkers	12.18				.06
Line Construction:					
Group 1	10.12	.45	7%	a	½%
Group 2	6.58	.45	7%	a	½%
Group 3	6.78	.45	7%	a	½%
Group 4	8.10	.45	7%	a	½%
Group 5	5.57	.45	7%	a	½%
Sheet metal worker	12.20		.55		.01
Tile setter	10.71		.55		
Decision #IA77-4233-Mod. #3					
(42 FR-53020-September 30, 1977)					
Story County, Iowa					
Change:					
Boilermakers	12.30	.85	1.00		.02
Carpenters	9.88		.75		
Cement Mason	10.56		.80		.04
Line Construction:					
Group 1	10.12	.45	7%	a	½%
Group 2	6.58	.45	7%	a	½%
Group 3	6.78	.45	7%	a	½%
Group 4	8.10	.45	7%	a	½%
Group 5	5.57	.45	7%	a	½%
Millwrights	10.23	.85	.75		

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
Decision #IA77-4227-Mod. 4					
(42 FR-53009-September 30, 1977)					
Dubuque County, Iowa					
Change:					
Boilermakers	12.30	.85	1.00		.02
Carpenters	10.18		.37		35.00 P/yr
Electricians	11.50	.65	3%+.35		3/4%
Glaziers	10.59	.70	1.00		.02
Laborers:					
Group 1	8.33	.30	.30		
Group 2	8.43	.30	.30		
Group 3	8.58	.30	.30		
Millwrights	10.68		.37		35.00 P/yr
Painters:					
Brush or roller epoxy, paperhanging, tapers	8.95		.30		
Spraying, high work & steel	9.50		.30		
Piledrivermen	10.58		.37		35.00 P/yr
Roofers	9.55		.20		
Decision #IA77-4230-Mod. #6					
(42 FR-53015-September 30, 1977)					
Polk County, Iowa					
Change:					
Boilermakers	12.30	.85	1.00		.02
Carpenters	10.18	.60	.75		.08
Cement Masons	10.56		.80		.04
Electricians	12.53	.40	3%+.80		3/4%
Line Construction:					
Group 1	10.12	.45	7%	a	½%
Group 2	6.58	.45	7%	a	½%
Group 3	6.78	.45	7%	a	½%
Group 4	8.10	.45	7%	a	½%
Group 5	5.57	.45	7%	a	½%
Millwrights, Piledrivermen	10.53	.60	.75		.08
Soft Floor Layer	9.93	.60	.75		.08
Tile Marble, Terrazzo	11.69				

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
Decision #IA77-4231-Mod. #4					
42 FR-53017-September 30, 1977)					
Pottawattamie County, Iowa					
Change:					
Boilermaker	12.30	.85	1.00		.02
Carpenters	10.14	.50	.50	1.00	.03
Line Construction:					
Group 1	10.12	.45	7%	a	½%
Group 2	6.58	.45	7%	a	½%
Group 3	6.78	.45	7%	a	½%
Group 4	8.10	.45	7%	a	½%
Group 5	5.57	.45	7%	a	½%
Millwrights	10.39	.50	.50	1.00	.03
Piledrivermen	10.265	.50	.50	1.00	.03
Decision #IA77-4229-Mod. #5					
(42 FR-53013-September 30, 1977)					
Linn County, Iowa					
Change:					
Boilermakers	12.30	.85	1.00		.02
Cement Masons	10.78	.45			
Glaziers	10.59	.70	1.00		.02
Ironworkers	12.18				.06
Line construction:					
Group 1	10.12	.45	7%	a	½%
Group 2	6.58	.45	7%	a	½%
Group 3	6.78	.45	7%	a	½%
Group 4	8.10	.45	7%	a	½%
Group 5	5.57	.45	7%	a	½%
Sheet metal workers	12.20		.55		.01
Tile Setters	10.71		.55		

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
Decision #IA77-4234-Mod. #3					
(42 FR-53022-September 30, 1977)					
Webster County, Iowa					
Change:					
Boilermakers	12.30	.85	1.00		.02
Bricklayers	10.27		.40		
Carpenters	9.60				
Electricians	11.20	.40	3%		3/4%
Line Construction:					
Group 1	10.12	.45	7%	a	½%
Group 2	6.58	.45	7%	a	½%
Group 3	6.78	.45	7%	a	½%
Group 4	8.10	.45	7%	a	½%
Group 5	5.57	.45	7%	a	½%
Millwrights	9.85				
Decision #IA77-4232-Mod. #3					
(42 FR 53019-September 30, 1978)					
Scott County, Iowa					
Change:					
Boilermakers	12.30	.85	1.00		.02
Glaziers	10.59	.70	1.00		.02
Laborers					
Group 1	9.72	.40	1.00		.035
Group 2	9.97	.40	1.00		.035
Group 3	10.22	.40	1.00		.035
Painters					
Brush, Roller	10.27	.55	.85		.18
spray, structural steel	10.77	.55	.85		.18

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
Decision #IA77-4235-Mod. #5					
(42 FR-53024-September 30, 1977)					
Woodbury County, Iowa					
Boilermakers	12.30	.85	1.00		.02
Electricians:					
With 15 mile radius of Sioux City and all electrical contracts over $300,000	12.01	.50	3%+.60		3/4%
Line construction:					
Group 1	10.12	.45	7%	a	½%
Group 2	6.58	.45	7%	a	½%
Group 3	6.78	.45	7%	a	½%
Group 4	8.10	.45	7%	a	½%
Group 5	5.57	.45	7%	a	½%
Plumbers	11.52	.74	.80		.10
Sheetmetal workers	10.79	.50	.55		.04
Decision #IA77-4226-Mod. #4					
(42 FR-53007-September 30, 1977)					
Des Moines County, Iowa					
Change:					
Boilermakers	12.30	.85	1.00		.02
Carpenters	10.70	.75	.90		.04
Electricians	12.45	.55	3%		3/4%
Glaziers	10.59	.70	1.00		.02
Ironworkers	11.50	.55	.90		.06
Line Construction:					
Group 1	10.12	.45	7%	a	½%
Group 2	6.58	.45	7%	a	½%
Group 3	6.78	.45	7%	a	½$
Group 4	8.10	.45	7%	a	½%
Group 5	5.57	.45	7%	a	½%
Millwrights	11.40	.75	.90		.04
Painters					
Group 1	9.70		.25		
Group 2	10.40		.25		
Group 3	9.80		.25		
Group 4	10.70		.25		
Group 5	10.20		.25		
Piledrivermen	11.40	.75	.90		.04
Roofers	10.12				

Decision No. NC78-1061 - Mod. #1 (43 FR 29453 - July 7, 1978) Statesville, North Carolina Change: Page 2: Decision No. NC78-1061	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
DECISION NO. OK77-4060 - Mod. #2 (42 FR 13786 - March 11, 1977) Comanche County, Oklahoma CHANGE: PAINTERS, brush	$6.00		.25	.70	.05

Decision PA77-3122 - Mod. # 7 (42 FR 45614 - Sept., 9, 1977) Cumberland, Dauphin, Perry, Juniata, New Cumberland Depot in York County, Pennsylvania Change: Piledrivermen Modification # 2, dated March 10, 1978, in 43 FR 10159 to read Modification # 3; Modification # 3, dated May 5, 1978, in 43 FR 19548, to read Modification # 4; Modification # 4, dated May 26, 1978, in 43 FR 22870, to read Modification # 5; Modification # 5, dated July 7, 1978, in 43 FR 29424, to read Modification # 6	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
	$11.12	2.33	1.35	a	.12
DECISION #PA78-3037 - Mod. # 1 (43 FR 17239 - April 21, 1978) Blair County, Pennsylvania Add: Elevator Constructors Elevator Constructors Helpers Elevator Constructors Helpers (Prob.)	$10.98 7.69 5.49	.745 .745	.56 .56	a+b a+b	.025 .025

Footnotes:

a. Employers contributes 8% basic hourly rate for 5 years or more of service as vacation pay credit.

b. Paid Holidays: New Year's Day; Memorial Day; Independence Day; Labor Day; Thanksgiving Day; Christmas Day, plus the Friday after Thanksgiving Day.

DECISION NO. TN78-1058 - Mod. #1
(43 FR 29464 - July 7, 1978)
Hamilton, Marion, Polk, and Rhea Counties, Tennessee

CHANGE:

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
Bricklayers, Stone masons, Cement masons, & Plasterers:					
Polk County Only	$ 8.90				
Glaziers	8.81			1%	
Laborers:					
Group A	6.60	.25	.30		
Group B	6.70	.25	.30		
Group C	6.80	.25	.30		
Group D	6.90	.25	.30		
Group E	6.95	.25	.30		
Group F	7.25	.25	.30		
Group G	7.30	.25	.30		
Free Air Shafts & Tunnels					
Group H	7.00	.25	.30		
Group I	7.15	.25	.30		
Group J	7.40	.25	.30		
(Note, see page 2 of Mod. for classifications definitions.)					
Sheet metal workers:					
Hamilton County	10.23	.61	.77		.01¼
All other Counties	10.73	.61	.77		.01¼

ADD:

Line Construction:					
Groundmen	7.45	.70	9%		½ of 1%

DECISION NO. TN78-1058 - Mod. #1 (Cont'd)

CLASSIFICATIONS DEFINITIONS

Group A - General laborers, concrete laborers, carpenter tenders, window and floor cleaners, and flagman on road and street crossings, form strippers, handling of rope to clam bucket, grout men, laborers working on demolition work, handling, cleaning and pulling of nails from materials.

Group B - Powder man helpers, tenders to all trowel trades and terraza work, carrying re-inforced steel, operating motorized wheel barrows, doping and painting of pipe, railroad track laborers, snake men on pipe work.

Group C - Sanitary and storm pipe layers or any other pipe outside of foundation, grade checker, yarner, and pot man, steel form setters, mortar mixers, by hand or machine, power saw operators, jackhammer operator, air tool operators, regular air tamp operators, wacker tamp operator, chipping hammer operator, hand operated ditching machine operator, ditching machine operator, concrete grinder, floor sweeping machine operator, concrete buffer and grinder power operator, persons working with concrete pumping machines, vibrator operators, and air spade operators.

Group D - Asphalt raker, wagon drill operator, sand blasting, track drill operators, concrete saw operator, using cutter torch or burner on demolition work, flagging of rigs.

Group E - Barco tamp operator and specially designed tamp operator, black top or concrete curbing machine operator, and pavement breaker operator.

Group F - Powderman, motorized post hole digger operator and terraza machine grinder.

Group G - Pneumatic concrete gun operator and nozzleman.

Group H - Tunnel laborer.

Group I - Chuck tender, top lander on shaft work.

Group J - Tunnel miner, including men required to go down in pier hole drilled machines.

SUPERSEDEAS DECISION

STATES: Arkansas, Louisiana, Mississippi and Tennessee
DECISION NO.: AR78-4074 DATE: Date of Publication
Supersedes Decision No. AR78-4070, dated June 30, 1978, in 43 FR 28722.
DESCRIPTION OF WORK: For construction of all river, harbor and floor control work on the Mississippi River and tributaries (excluding the metropolitan areas of Vicksburg, Greenville and Natchez, Mississippi; Pine Bluff, Little Rock and Ft. Smith, Arkansas; Memphis, Tennessee and New Orleans, Baton Rouge, Alexandria, Monroe and Shreveport, Louisiana and any contracts for any phase of construction of a lock or dam.)

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
CARPENTERS	$ 4.50				
LABORERS:					
Unskilled	3.15				
Revetment and dikes	3.15				
Chain saw operator or filer	3.50				
Air tool operator	3.50				
Powderman	4.50				
POWER EQUIPMENT OPERATORS:					
Pile driver operator, mechanic (heavy equipment), cranes, derricks, draglines, welder, power shovels and backhoes, mixer (concrete, 21 cu. ft. & over), asphalt plant operator, trenching machine (over 18")	6.35				.05
Bulldozer (finisher, push cat & on barges), motor patrol finisher, scraper and like equipment, front end loader, backhoe (tractor mounted), asphalt finisher or spreading machine, well point system operator, self-propelled loader (conveyor type)	5.30				.05
Fireman (heavy construction), pile driver, leadsman, winchman	4.50				.05
Asphalt plant dryer operator, asphalt distributor, asphalt roller, bulldozer (rough, including disc, plow or roller), motor patrol (haul roads), trenching machine (18" and under), self-propelled roller (except asphalt), end dump equipment (off highway), mixer (concrete up to 21 cu. ft), bottom dump euclids (and like equipment)	4.40				.05
Oiler, pump, mechanic helper, greaser, welder helper, tractor (farm type including disc, plow or roller)	3.95				.05
TRUCK DRIVERS:					
1½ tons or less	3.50				
Over 1½ tons	4.00				

SUPERSEDEAS DECISION

STATE: Arizona COUNTY: Pima
DECISION NUMBER: AZ78-5116 DATE : Date of PUblication
Supersedes Decision No. AZ77-5026 dated June 17, 1977, in 42 FR 31070
DESCRIPTION OF WORK: Residential Construction (consisting of single family homes and garden type apartments up to and including 4 stories)

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
ASBESTOS WORKERS	$ 11.94	.50	$ 1.20		.02
BOILERMAKERS	13.625	1.075	1.00	.75	.02
BRICKLAYERS; Stonemasons:					
Zone A (0-15 miles from Tucson)	11.22	1.00	.90		.06
Zone B (15-30 miles from Tucson)	11.59	1.00	.90		.06
Zone C (30-40 miles from Tucson)	11.96	1.00	.90		.06
Zone D (40 miles and over from Tucson)	12.72	1.00	.90		.06
CARPENTERS:					
Carpenters; Drywall Applicator	9.785	.845	.955		.025
Piledrivermen; Floor Layers (finish)	10.07	.845	.955		.025
Millwrights	10.20	.845	.955		.025
CEMENT MASONS	10.22	.85	.85		.05
ELECTRICIANS:					
Zone A (0-16 miles from City Hall in Tucson)	9.39	8.4%	3%		1/2%
Zone B (26-32 miles from City Hall in Tucson)	9.89	8.4%	3%		1/2%
Zone C (32-48 miles from City Hall in Tucson)	10.39	8.4%	3%		1/2%
Zone D (48 miles and over from City Hall in Tucson)	10.89	8.4%	3%		1/2%
ELEVATOR CONSTRUCTORS	11.775	.745	.56	3%+a	.025
ELEVATOR CONSTRUCTORS' HELPERS	70%JR	.745	.56	3%+a	.025
ELEVATOR CONSTRUCTORS' HELPERS (PROB.)	50%JR				
GLAZIERS	9.42	.70	.30		.01
IRONWORKERS	11.48	1.24	2.22		.08
LATHERS:					
Zone A (0-30 miles from Tucson)	9.28	.35	.40		
Zone B (30-40 miles from Tucson)	9.78	.35	.40		
Zone C (40-50 miles from Tucson)	10.03	.35	.40		
Zone D (Area outside Zone C)	10.78	.35	.40		
MARBLE SETTERS; Terrazzo Workers; Tile Setters	9.42	.70	.75		

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
PAINTERS, Brush:					
Zone A (0-30 miles from Tucson Post Office)	$ 8.78	.67	.35		.04
Zone B (31-40 miles from Tucson Post Office)	9.53	.67	.35		.04
Zone C (41-50 miles from Tucson Post Office)	10.03	.67	.35		.04
Zone D (51 miles and over from Tucson Post Office)	10.78	.67	.35		.04
PAINTERS; Structural Steel, Brush:					
Zone A (0-30 miles from Tucson Post Office)	9.78	.67	.35		.04
Zone B (31-40 miles from Tucson Post Office)	10.53	.67	.35		.04
Zone C (41-50 miles from Tucson Post Office)	11.03	.67	.35		.04
Zone D (51 miles and over from Tucson Post Office)	11.78	.67	.35		.04
PLASTERERS:					
Zone A (0-30 miles from Tucson Post Office)	8.57	.35	.60		
Zone B (30-40 miles from Tucson Post Office)	9.07	.35	.60		
Zone C (40-50 miles from Tucson Post Office)	9.32	.35	.60		
Zone D (50 miles and over from Tucson Post Office)	10.07	.35	.60		
PLASTERERS' TENDERS	9.16	.85	.85		.10

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
PLUMBERS; Steamfitters:					
FREE ZONE 0-15 miles					
The "Free Zone" (Zone I shall be 15 road miles from the stated base points in Flagstaff, Yuma, Tucson and Douglas. The "Free Zone" from Phoenix shall be 15 miles radius from the stated base point. In addition, all areas within the City limits of Phoenix, Chandler, Scottsdale, Tempe, Glendale, Mesa, Kingman, Havasu City, Prescott, Winslow and Holbrook will be included as Free Zones). Any work contracted from outside of these zones will be determined from the Phoenix and Tucson basing points.	$ 12.24	.75	$ 1.35		.10
Zone II (15-30 miles)	12.59	.75	1.35		.10
Zone III (30-40 miles)	13.01	.75	1.35		.10
Zone IV (40 miles and over)	14.31	.75	1.35		.10
ROOFERS:					
Zone A (0-44 miles from Tucson)	8.97	.845	.20		.02
Zone B (over 44 miles from Tucson)	10.72	.845	.20		.02
SHEET METAL WORKERS:					
Zone A (0-22 miles from Tucson)	9.90	3%+1.04	1.91		.04
Zone B (22-45 miles from Tucson)	10.85	3%+1.04	1.91		.04
Zone C (Over 45 miles from Tucson)	12.40	3%+1.04	1.91		.04
SOFT FLOOR LAYERS	9.25	.38			
SPRINKLER FITTERS	12.29	.65	.95		.08
TERRAZZO WORKERS; TILE SETTERS; MARBLE SETTERS	9.27	.90	.85		

FOOTNOTE:

a. Employer credits 4% basic hourly rate of employee with over 5 years' service, 2% basic hourly rate from 6 months' to 5 years' service to Vacation Fund. 6 Paid HOlidays: A through F.

PAID HOLIDAYS:

A-New Year's Day; B-Memorial Day; C-Independence Day; D-Labor Day; E-Thanksgiving Day; F-Christmas Day.

LABORERS	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
Group 1:	$ 8.01	.85	.85		.10
Group 2:	8.14	.85	.85		.10
Group 3:	8.28	.85	.85		.10
Group 4:	8.39	.85	.85		.10
Group 5:	8.56	.85	.85		.10
Group 6:	8.935	.85	.85		.10
Group 7:	9.565	.85	.85		.10
POWER EQUIPMENT OPERATORS (Except Piledriving & Steel Erection)					
Group 1:	8.60	.95	.90		.06
Group 2:	8.97	.95	.90		.06
Group 3:	9.43	.95	.90		.06
Group 4:	9.96	.95	.90		.06
Group 5:	10.49	.95	.90		.06
Group 5A:	10.80	.95	.90		.06
Group 6:	11.13	.95	.90		.06
Group 7:	11.73	.95	.90		.06
TRUCK DRIVERS					
Group 1:	8.19	.85	.85		.06
Group 2:	8.32	.85	.85		.06
Group 3:	8.54	.85	.85		.06
Group 4:	8.89	.85	.85		.06
Group 5:	9.05	.85	.85		.06
Group 5A:	9.23	.85	.85		.06
Group 6:	9.37	.85	.85		.06
Group 7:	9.78	.85	.85		.06
Group 8:	10.295	.85	.85		.06
Group 8A:	10.95	.85	.85		.06
Group 8B:	10.64	.85	.85		.06
Group 8C:	8.75	.85	.85		.06

LABORERS

Group 1: All Helpers not herein separately classified; Cesspool Diggers and installers; Chat Box Man; Checker, tool dispatcher; Concrete dump manbelt, pipe and/or hoseman; Dumpman and/or spotter; Fence builder, guard rail builder highway; Form strippers; Labor, general or construction; Landscape gardener and nurseryman; Packing rod steel and pans; Rip rap stoneman; Astro turf layer; Cleanup, Bull gang; Trackman-railroad

Group 2: Cement finisher tender; Concrete curer (impervious membrane); Cutting torch operator; Fine grader (highway, engineering and sewer work only); Kettleman - Tarman; Power type concrete buggy

Group 3: Bander; Chucktender (except tunnel); Creosote tieman; Guinea chaser; Powderman helper; Rip-rap stone paver; Sandblaster (pot tender); Spiker and wrenchers

Group 4: Cement dumpers (Skip-type mixer or handling bulk cement); Chain saw machines (on clearing and grubbing); Concrete vibrating machines; Cribber and shorer (except tunnel); Floor sanders concrete; Hydraulic jacks, and similar mechanical tools not separately classified herein; Operators and tenders of pneumatic and electric tools; Pipe caulker and/or backup man (pipeline); Pipe wrapper; Pneumatic gopher; Rigger/Signalman (pipeline)

Group 5: Air and water wash-out nozzleman; Asphalt rakers and ironers; Driller; Grade setter (pipeline); Hand guided trencher and similar operated equipment; Jackhammer and/or pavement breakers; Pipelayers (including but not limited to non-metallic, transite and plastic pipe, water pipe, sewer pipe, drain pipe, underground tile and conduit); Rock slinger; Scaler (using Bos'ns chairs or safety belt); Tampers (mechanical - all types); Precast manhole erector

Group 6: Concrete Cutting Torch; Concrete saw (hand guided); Driller, (core, diamond, wagon or air track); Drill doctor and/or air tool repairman; Gunman and mixerman (gunite); Sandblaster (nozzleman)

Group 7: Concrete Road Form Setter; Gunite nozzleman or roadman; Drillers, Joy Mustang, PR 143, 200 Gardner-Denver, Hydrasonic; Powderman; Scaler (drillers); Welders and/or pipelayers installing process piping; Form setter and/or builder

POWER EQUIPMENT OPERATORS
(Except Piledriving and Steel Erection)

Group 1: Air compressor operator; Field equipment servicemen helper; Heavy duty repair helper; Heavy duty welder helper; Oiler; Pump operator

Group 2: Conveyor operator; Generator operator - portable; Power grizzly operator; Self-propelled chip spreading machine - convoyor operator; Watch fireman; Welding machine operator - gasoline and diesel power

Group 3: Concrete mixer operator - skip type; Dinky operator - (under 20 tons wt.); Driver-moto paver, Slurry seal machine, and similar type equipment; Motor crane driver; Power sweeper operator - self-propelled; Ross carrier or fork lift operator; Skip loader operator - all types with rated capacity 1-1/2 cu. yds. or less; Wheel type tractor operator (Ford, Ferguson, or similar type) with attachments such as fresno, push blade, post hole auger, mower, etc., excluding compacting equipment

Group 4: A-Frame boom truck or winch truck operator; Asphalt plant firemen; Elevator hoist operator (including Tuskey hoist or similar type); Grade checker (excluding civil engineer); Multiple power concrete saw operator; Pavement breaker, mechanical compactor operator, power propelled; Roller operator - all types - except as otherwise classified; Screed operator; Self-propelled chip spreading machine operator (including Slurry seal machine operator) Stationary pipewrapping and cleaning machine operator; Tugger operator

Group 5: Aggregate plant operator (including crushing, screening and sand plants, etc.); Asphalt plant mixer operator; Beltcrete machine; Boring machines operator; Concrete mechanical tamping, spreading or finishing machine (including Clary, Johnson or similar types); Concrete pumps operator; Concrete batch plant operator, all types and sizes; Conductor, brakeman, or handler; Drilling machine, including water wells; Elevating grader operator - all types and sizes (except as otherwise classfied); Field equipment serviceman; Highline cableway signalman; Kolman belt loader operator or similar, with belt width 48" or over; Locomotive engineer (including Dinky - 20 tons wt. and over); Moto-paver and similar type equipment operator; Operating engineer rigger; Pneumatic-tired scraper operator (Turnapull, Euclid, Cat, D-W, Hancock and similar equipment) up to and including 12 cu. yds.; Power jumbo form setter operator; Pressure grout machine operator (as used in heavy engineering construction); Road oil mixing machine operator; Roller operator - on all types asphalt pavement; Self-propelled compactor, with blade; Skip loader operator - all types with rated capacity over 1-1/2 but less than 4 cu. yds.; Slip form operator (power driven lifting device for concrete forms); Soil cement road mixing machine operator - single pass type. Stationary Central generating plant operator - rated 300 K.W. or more; Surface heater and planer operator; Traveling pipewrapping machine operator

POWER EQUIPMENT OPERATORS (Cont'd)
(Except Piledriving and Steel Erection)

Group 5-A: Heavy duty mechanic and/or welder; Pneumatic tired scraper, all sizes and types over 12 cu. yds. up to and including 45 cu. yds. MRC (Turnapull, Euclid, Cat, D-W, Hancock and similar equipment); Tractor operator (Pusher, Bulldozer, Scraper) up to 400 net horsepower rating; Trenching machine operator

Group 6: Auto-Grade machine (CMI and similar equipment); Boring machine operator (including Mole, Badger and similar type); Concrete Mixer operator-paving type, and mobile mixer; Concrete pump operator with boom attachment (truck mounted); Crane operator - crawler and pneumatic type, under 100 ton capacity MRC; Crawler type tractor operator - with boom attachment; Derrick operator; Forklift operator for hoisting personnel; Grade-all operator; Helicopter hoist; Highline cableway operator (less than 20 tons rated capacity); Mass excavator operator (150 Bucyrus Erie and similar types); Mechanical hoist operator (two or more drums); Motor grade operator - any type power blade; Motor grade operator with elevating grader attachment; Mucking machine operator; Overhead crane operator; Piledriver engineer (portable, stationary or skid rig); Pneumatic-tired scraper operator - all sizes and types (Turnapull, Euclid, Cat, D-W, Hancock and similar equipment over 45 cu. yds., MRC); Power driven ditch lining or ditch trimming machine operator; Skip loader operator - all types with rated capacity 4 cu. yds., but less than 8 cu. yds.; Slip form paving machine operator (including Gunnert, Zimmerman and similar types); Specialized power digger operator - attached to wheel-type tractor; Tower crane (or similar type) operator; Tractor operator (Pusher, Bulldozer, Scraper (400 net horsepower and over); Tugger operator (two or more); Universal equipment operator - Shovel, Backhoe, Dragline, Clamshell, etc. up to 8 cu. yds.

Group 7: Crane operator - pneumatic or crawler (100 ton hoisting capacity and over MRC rating); Helicopter pilot - FAA qualified when used in construction work; Highline cableway operator, over 20 ton rated capacity and using traveling head and tail tower; Remote control earth moving equipment operator; Skip loader operator - all types with rated capacity of 8 cu. yds. or more; Universal equipment - Shovel, Backhoe, Dragline, Clamshell, etc., 8 cu. yds. and over

TRUCK DRIVERS

Group 1: Teamsters; Pickups; Station Wagons; Manhaul driver

Group 2: Dump or flatrack (2 or 3 axle); Water truck (under 2500 gallons); Buggymobile (1 cu. yd. or less); Tireman; Bus drivers, ambulance driver, self-propelled street sweeper; Warehouseman

Group 3: Dump or flatrack (4 axle); Dumptor or dumpster (less than 7 cu. yds.); Water truck (2500 gallons but less than 4000 gallons)

Group 4: Dumptor or dumpster (7 cu. yds. but less than 16 cu. yds.); Dump or flatrack (5 axle); Water truck (4000 gallons and over); Slurry type equipment or leverman; Flaherty spreader or similar type equipment or leverman; Transit mix (8 cu. yds. or less)

Group 5: Dump or flatrack (6 axle); Transit mix (over 8 cu. yds. but less than 10.5 cu. yds.); Rock truck (i.e. Dart, Euclid and other similar type end dumps, single unit less than 16 cu. yds.)

Group 5-A: Oil tanker or spreader and/or bootman, retortman or leverman

Group 6: Transit mix (over 10.5 cu. yds. but less than 14 cu. yds.); Ross Carrier; Fork lift or lift truck; Hydro lift, Swedish crane Iowa 300 and similar types; Concrete pump (when integral part of transit mix truck); Dump or flat rack (7 axle)

Group 7: Dump or Flatrack (8 axles)

Group 8: Off-highway equipment driver including but not limited to: 2 or 4 wheel power unit, i.e., Cat, DW Series, Euclid, International and similar type equipment, transporting material when top loaded or by external means including pulling water tanks, fuel tanks or other applications under Teamster Classifications; Rock trucks (Dart, Euclid, or other similar end dump types) 16 cu. yds. and over; Eject-alls; Dumptor or dumpster (16 cu. yds. and over); Dump or flatrack (9 axles)

Group 8A: Heavy duty mechanic/welder; Body and fender man

Group 8B: Field equipment serviceman or fuel truck driver

Group 8C: Heavy duty mechanic/welder helper.

SUPERSEDEAS DECISION

STATE: Arizona COUNTY: Maricopa
DECISION NUMBER: AZ78-5115 DATE: Date of Publication
Supersedes Decision No. AZ77-5025 dated June 17, 1977, in 42 FR 31065
DESCRIPTION OF WORK: Residential Construction (consisting of single family homes and garden type apartments up to and including 4 stories)

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
ASBESTOS WORKERS	$ 11.94	.50	$ 1.20		.02
BOILERMAKERS	13.625	1.075	1.00	.75	.02
BRICKLAYERS; Stonemasons:					
Zone A (0-25 miles from City Hall of Phoenix; Flagstaff and Yuma)	11.77	.65	.90		.09
Zone B (25-40 miles from City Hall of Phoenix; and Williams AFB)	12.71	.65	.90		.09
Zone C (40-70 miles from City Hall of Phoenix)	13.30	.65	.90		.09
Zone D (70-100 miles from City Hall of Phoenix)	13.89	.65	.90		.09
Zone E (100-200 miles from City Hall of Phoenix)	14.36	.65	.90		.09
Zone F (200 miles and over from City Hall of Phoenix)	15.30	.65	.90		.09
CARPENTERS:					
Carpenters; Drywall Applicator	9.785	.845	.955		.05
Piledrivermen; Floorlayers (finish)	10.07	.845	.955		.05
Millwrights	10.20	.845	.955		.05
CEMENT MASONS:					
Northern portion	10.21	.95	1.30		.05
Southern portion	10.22	.85	.85		.05
DRYWALL:					
(from Courthouse in Phoenix, Mesa, including Williams AFB and Luke AFB):					
Zone A (0-40 miles)	10.21	.59	.50		.07
Zone B (41-60 miles)	11.21	.59	.50		.07
Zone C (61 miles and over)	12.46	.59	.50		.07
Texture Spraymen:					
Zone A (0-40 miles)	9.81	.59	.50		.07
Zone B (41-60 miles)	10.81	.59	.50		.07
Zone C (51 miles and over)	12.06	.59	.50		.07

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
ELECTRICIANS:					
Zone A (Beginning at the northeast corner, a line extending southward on Bush Highway to McKellips Road; a line extending east on McKellips Road to a point one mile east of the intersection of State Highway 88 and U. S. 60 and 70 near Apache Junction; southward to Baseline Road; west on Baseline Road to the intersection of Baseline Road and Ellsworth Road; South on Ellsworth Road to Hunt Highway; West on Hunt Highway to Powers Road; a line extending south on Powers Road five miles, then extending straight west to a point five miles west of Interstate 10, then northwest on a line parallel with Interstate 10 to intersect with Pecos Road; West on Pecos to intersect with Cotton Lane. North on Cotton Lane to Beloat Rosd. West on Beloat Road to Airport Road. North on Airport Road in a straight line to intersect Waddell Road. East on Waddell Road to intersect with Cotton Lane. North on Cotton Lane to Deer Valley Drive and east on Deer Valley Drive to intersection with Bush Highway and including Luke and Williams Air Force Bases.)					
Electricians	$ 12.78	.96	3%+.88		3/4%
Cable Splicers	12.64	.96	3%+.88		3/4%

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
ELECTRICIANS: (Cont'd)					
Zone B (Area outside of Zone A and bounded by a line formed by measuring sixteen (16) road miles from the outer boundaries of an area enclosed by the following boundaries; Power Road on the east from Hunt Highway on the south to one mile south of Pinnacle Peak Road on the north. One mile south of Pinnacle Peak Road to Cotton Lane on the west. Cotton Lane to Pecos Road on the south. Pecos Road to Price Road and from Price Road to Hunt Highway on the south. Hunt Highway to Powers Road on the east.)					
Electricians	$ 15.02	.96	3%+.88		3/4%
Cable Splicers	14.85	.96	3%+.88		3/4%
Zone C (Outside edge of Zone B and extend to the outside limits of the Union's jurisdiction)					
Electricians	16.17	.96	3%+.88		3/4%
Cable Splicers	15.99	.96	3%+.88		3/4%
ELEVATOR CONSTRUCTORS	11.775	.745	.56	3%+a	.025
ELEVATOR CONSTRUCTORS' HELPERS	70%JR	.745	.56	3%+a	.025
ELEVATOR CONSTRUCTORS' HELPERS (PROB.)	50%JR				
GLAZIERS	9.42	.70	.30		.01
IRONWORKERS	11.48	1.24	2.22		.08

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
PAINTERS:					
Zone A (0-40 miles from Court House in Phoenix, Mesa and including Luke and Williams Air Force Bases):					
Brush; Tapers	$ 9.85	.60	.40		.08
Spray; Paperhangers	10.10	.60	.40		.08
Zone B (41-60 miles from Court House in Phoenix)					
Brush; Tapers	10.85	.60	.40		.08
Spray; Paperhangers	11.10	.60	.40		.08
Zone C (61 miles and over from Court House in Phoenix)					
Brush; Tapers	12.10	.60	.40		.08
Spray; Paperhangers	12.35	.60	.40		.08
PLASTERERS (Northern 3/4 of County):					
Zone A (0-35 miles from Phoenix)	9.045	.60	.85		.035
Zone B (35-60 miles from Phoenix)	9.795	.60	.85		.035
Zone C (60 miles and over from Phoenix)	10.67	.60	.85		.035
PLASTERERS (Southern 1/4 of County):					
Zone A (0-30 miles from Tucson)	8.57	.35	.60		
Zone B (30-40 miles from Tucson)	9.07	.35	.60		
Zone c (40-50 miles from Tucson)	9.32	.35	.60		
Zone D (50 miles and over from Tucson)	10.07	.35	.60		
PLASTERERS' TENDERS	9.16	.85	.85		.10

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
PLUMBERS; Steamfitters:					
FREE ZONE 0-15 miles					
The "Free Zone" (Zone I shall be 15 road miles from the stated base points in Flagstaff, Yuma, Tucson and Douglas. The "Free Zone" from Phoenix shall be 15 mile radius from the state base point. In addition, all areas within the City limits of Phoenix, Chandler, Scottsdale, Tempe, Glendale, Mesa, Kingman, Havasu City, Prescott, Winslow and Holbrook will be included as Free Zones. Any work contracted from outside of these zones will be determined from the Phoenix and Tucson basing points:					
Plumbers; Steamfitters	$ 12.24	.75	$ 1.35		.10
Zone II (15-30 miles)	12.59	.75	1.35		.10
Zone III (30-40 miles)	13.01	.75	1.35		.10
Zone IV (40 miles and over)	14.31	.75	1.35		.10
ROOFERS	9.66	.845	.20		.02
SHEET METAL WORKERS:					
Zone I (0-25 miles excluding Luke and Williams AFB)	10.83	3%+.80	1.30		.10
Zone II (25-50 miles including Luke and Williams AFB)	11.48	3%+.80	1.30		.10
Zone III (50 miles and over)	13.33	3%+.80	1.30		.10
SOFT FLOOR LAYERS:					
Zone A (0-40 miles from Court House in Phoenix and including Luke and Williams AFB)	9.21	.59	.12		.12
Zone B (41-60 miles from Court House in Phoenix)	10 21	.59	.12		.12
Zone C (61 miles and over)	10 71	.59	.12		.12
SPRINKLER FITTERS	12.29	.65	.95		.08

FOOTNOTE:
a. Employer contributes 4% of basic hourly rate for 5 years' service and 2% of basic hourly rate for 6 months to 5 years' service as Vacation Pay Credit. Six Paid Holidays: A through F.

PAID HOLIDAYS:
A-New Year's Day; B-Memorial Day; C-Independence Day; D-Labor Day; E-Thanksgiving Day; F-Christmas Day.

LABORERS	Basic Hourly Rates	Fringe Benefits Payments			
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Group 7:	9.565	.85	.85		.10
POWER EQUIPMENT OPERATORS (Except Piledriving & Steel Erection)					
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Group 2:	8.97	.95	.90		.06
Group 3:	9.43	.95	.90		.06
Group 4:	9.96	.95	.90		.06
Group 5:	10.49	.95	.90		.06
Group 5A:	10.80	.95	.90		.06
Group 6:	11.13	.95	.90		.06
Group 7:	11.73	.95	.90		.06
TRUCK DRIVERS					
Group 1:	8.19	.85	.85		.06
Group 2:	8.32	.85	.85		.06
Group 3:	8.54	.85	.85		.06
Group 4:	8.89	.85	.85		.06
Group 5:	9.05	.85	.85		.06
Group 5A:	9.23	.85	.85		.06
Group 6:	9.37	.85	.85		.06
Group 7:	9.78	.85	.85		.06
Group 8:	10.295	.85	.85		.06
Group 8A:	10.95	.85	.85		.06
Group 8B:	10.64	.85	.85		.06
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LABORERS

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Group 2: Cement finisher tender; Concrete curer (impervious membrane); Cutting torch operator; Fine grader (highway, engineering and sewer work only); Kettleman - Tarman; Power type concrete buggy

Group 3: Bander; Chucktender (except tunnel); Creosote tieman; Guinea chaser; Powderman helper; Rip-rap stone paver; Sandblaster (pot tender); Spiker and wrenchers

Group 4: Cement dumpers (Skip-type mixer or handling bulk cement); Chain saw machines (on clearing and grubbing); Concrete vibrating machines; Cribber and shorer (except tunnel); Floor sanders concrete; Hydraulic jacks, and similar mechanical tools not separately classified herein; Operators and tenders of pneumatic and electric tools; Pipe caulker and/or backup man (pipeline); Pipe wrapper; Pneumatic gopher; Rigger/Signalman (pipeline)

Group 5: Air and water wash-out nozzleman; Asphalt rakers and ironers; Driller; Grade setter (pipeline); Hand guided trencher and similar operated equipment; Jackhammer and/or pavement breakers; Pipelayers (including but not limited to non-metallic, transite and plastic pipe, water pipe, sewer pipe, drain pipe, underground tile and conduit); Rock slinger; Scaler (using Bos'ns chairs or safety belt); Tampers (mechanical - all types); Precast manhole erector

Group 6: Concrete Cutting Torch; Concrete saw (hand guided); Driller, (core, diamond, wagon or air track); Drill doctor and/or air tool repairman; Gunman and mixerman (gunite); Sandblaster (nozzleman)

Group 7: Concrete Road Form Setter; Gunite nozzleman or roadman; Drillers, Joy Mustang, PR 143, 200 Gardner-Denver, Hydrasonic; Powderman; Scaler (drillers); Welders and/or pipelayers installing process piping; Form setter and/or builder

POWER EQUIPMENT OPERATORS
(Except Piledriving and Steel Erection)

Group 1: Air compressor operator; Field equipment servicemen helper; Heavy duty repair helper; Heavy duty welder helper; Oiler; Pump operator

Group 2: Conveyor operator; Generator operator - portable; Power grizzly operator; Self-propelled chip spreading machine - conveyor operator; Watch fireman; Welding machine operator - gasoline and diesel power

Group 3: Concrete mixer operator - skip type; Dinky operator - (under 20 tons wt.); Driver-moto paver, Slurry seal machine, and similar type equipment; Motor crane driver; Power sweeper operator - self-propelled; Ross carrier or fork lift operator; Skip loader operator - all types with rated capacity 1-1/2 cu. yds. or less; Wheel type tractor operator (Ford, Ferguson, or similar type) with attachments such as fresno, push blade, post hole auger, mower, etc., excluding compacting equipment

Group 4: A-Frame boom truck or winch truck operator; Asphalt plant firemen; Elevator hoist operator (including Tuskey hoist or similar type); Grade checker (excluding civil engineer); Multiple power concrete saw operator; Pavement breaker, mechanical compactor operator, power propelled; Roller operator - all types - except as otherwise classified; Screed operator; Self-propelled chip spreading machine operator (including Slurry seal machine operator) Stationary pipewrapping and cleaning machine operator; Tugger operator

Group 5: Aggregate plant operator (including crushing, screening and sand plants, etc.); Asphalt plant mixer operator; Beltcrete machine; Boring machines operator; Concrete mechanical tamping, spreading or finishing machine (including Clary, Johnson or similar types); Concrete pumps operator; Concrete batch plant operator, all types and sizes; Conductor, brakeman, or handler; Drilling machine, including water wells; Elevating grader operator - all types and sizes (except as otherwise classfied); Field equipment serviceman; Highline cableway signalman; Kolman belt loader operator or similar, with belt width 48" or over; Locomotive engineer (including Dinky - 20 tons wt. and over); Moto-paver and similar type equipment operator; Operating engineer rigger; Pnuematic-tired scraper operator (Turnapull, Euclid, Cat, D-W, Hancock and similar equipment) up to and including 12 cu. yds.; Power jumbo form setter operator; Pressure grout machine operator (as used in heavy engineering construction); Road oil mixing machine operator; Roller operator - on all types asphalt pavement; Self-propelled compactor, with blade; Skip loader operator - all types with rated capacity over 1-1/2 but less than 4 cu. yds.; Slip form operator (power driven lifting device for concrete forms); Soil cement road mixing machine operator - single pass type; Stationary Central generating plant operator - rated 300 K.W. or more; Surface heater and planer operator; Traveling pipewrapping machine operator

POWER EQUIPMENT OPERATORS (Cont'd)
(Except Piledriving and Steel Erection)

Group 5-A: Heavy duty mechanic and/or welder; Pneumatic tired scraper, all sizes and types over 12 cu. yds. up to and including 45 cu. yds. MRC (Turnapull, Euclid, Cat, D-W, Hancock and similar equipment); Tractor operator (Pusher, Bulldozer, Scraper) up to 400 net horsepower rating; Trenching machine operator

Group 6: Auto-Grade machine (CMI and similar equipment); Boring machine operator (including Mole, Badger and similar type); Concrete Mixer operator-paving type, and mobile mixer; Concrete pump operator with boom attachment (truck mounted); Crane operator - crawler and pneumatic type, under 100 ton capacity MRC; Crawler type tractor operator - with boom attachment; Derrick operator; Forklift operator for hoisting personnel; Grade-all operator; Helicopter hoist; Highline cableway operator (less than 20 tons rated capacity); Mass excavator operator (150 Bucyrus Erie and similar types); Mechanical hoist operator (two or more drums); Motor grade operator - any type power blade; Motor grade operator with elevating grader attachment; Mucking machine operator; Overhead crane operator; Piledriver engineer (portable, stationary or skid rig); Pneumatic-tired scraper operator - all sizes and types (Turnapull, Euclid, Cat, D-W, Hancock and similar equipment over 45 cu. yds., MRC); Power driven ditch lining or ditch trimming machine operator; Skip loader operator - all types with rated capacity 4 cu. yds., but less than 8 cu. yds.; Slip form paving machine operator (including Gunnert, Zimmerman and similar types); Specialized power digger operator - attached to wheel-type tractor; Tower crane (or similar type) operator; Tractor operator (Pusher, Bulldozer, Scraper (400 net horsepower and over); Tugger operator (two or more); Universal equipment operator - Shovel, Backhoe, Dragline, Clamshell, etc. up to 8 cu. yds.

Group 7: Crane operator - pneumatic or crawler (100 ton hoisting capacity and over MRC rating); Helicopter pilot - FAA qualified when used in construction work; Highline cableway operator, over 20 ton rated capacity and using traveling head and tail tower; Remote control earth moving equipment operator; Skip loader operator - all types with rated capacity of 8 cu. yds. or more; Universal equipment - Shovel, Backhoe, Dragline, Clamshell, etc., 8 cu. yds. and over

TRUCK DRIVERS

Group 1: Teamsters; Pickups; Station Wagons; Manhaul driver

Group 2: Dump or flatrack (2 or 3 axle); Water truck (under 2500 gallons); Buggymobile (1 cu. yd. or less); Tireman; Bus drivers, ambulance driver, self-propelled street sweeper; Warehouseman

Group 3: Dump or flatrack (4 axle); Dumptor or dumpster (less than 7 cu. yds.); Water truck (2500 gallons but less than 4000 gallons)

Group 4: Dumptor or dumpster (7 cu. yds. but less than 16 cu. yds.); Dump or flatrack (5 axle); Water truck (4000 gallons and over); Slurry type equipment or leverman; Flaherty spreader or similar type equipment or leverman; Transit mix (8 cu. yds. or less)

Group 5: Dump or flatrack (6 axle); Transit mix (over 8 cu. yds. but less than 10.5 cu. yds.); Rock truck (i.e. Dart, Euclid and other similar type end dumps, single unit less than 16 cu. yds.)

Group 5-A: Oil tanker or spreader and/or bootman, retortman or leverman

Group 6: Transit mix (over 10.5 cu. yds. but less than 14 cu. yds.); Ross Carrier; Fork lift or lift truck; Hydro lift, Swedish crane Iowa 300 and similar types; Concrete pump (when integral part of transit mix truck); Dump or flat rack (7 axle)

Group 7: Dump or Flatrack (8 axles)

Group 8: Off-highway equipment driver including but not limited to: 2 or 4 wheel power unit, i.e., Cat, DW Series, Euclid, International and similar type equipment, transporting material when top loaded or by external means including pulling water tanks, fuel tanks or other applications under Teamster Classifications; Rock trucks (Dart, Euclid, or other similar end dump types) 16 cu. yds. and over; Eject-alls; Dumptor or dumpster (16 cu. yds. and over); Dump or flatrack (9 axles)

Group 8A: Heavy duty mechanic/welder; Body and fender man

Group 8B: Field equipment serviceman or fuel truck driver

Group 8C: Heavy duty mechanic/welder helper

SUPERSEDES DECISION

STATE: CONNECTICUT COUNTIES: *SEE BELOW
DECISION NUMBER: CT78-3055 DATE: DATE OF PUBLICATION
Supersedes Decision No.: CT78-3003 dated February 17, 1978 in 43 FR 7110.
DESCRIPTION OF WORK: Building Construction (including residential), heavy (excluding tunnel construction) and highway construction

*Fairfield, Litchfield and Windham Counties	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
ASBESTOS WORKERS: Fairfield Co.; Litchfield Co.: Barkhamsted, Bethlehem, Bridgewater, Cornwall, Goshen, Harwinton, Kent, Litchfield, Morris New Hartford, New Milford, Plymouth, Roxbury, Sharon, Torrington, Warren, Washington, Watertown, Winchester, Woodbury, & Thomaston; Windham Co.: Ashford, Chaplin, Eastford, Hampton, Scotland & Windham	$11.84	.75	1.16		
Litchfield Co.: Canaan, Colbrook Norfolk, N. Canaan & Salisbury; Windham Co.: Woodstock	10.65	.94	1.25		.01
Windham Co.: Brooklyn, Canterbury, Killingly, Plainfield, Pomfret, Putnam, Sterling & Thompson	10.28	1.00	1.18		
BOILERMAKERS	10.00	.60	10%		.01
BRICKLAYERS: Cement masons; Finishers; Marble masons; Plasterers, Stonemasons; Terrazzo Workers; Tile setters (Building Construction): Fairfield Co.: Shelton	9.70	.75	.75		
Fairfield Co.: Bridgeport, Easton, Fairfield, Monroe, Stratford & Trumbull	10.05	.75	.75		
Fairfield Co.: New Canaan, Norwalk, Ridgefield, Weston, Westport & Wilton	10.20	.75	.75		
Fairfield Co.: Darien & Stamford	10.62	.83	.25		
Fairfield Co.: Greenwich	10.45	.70	.70		.03
Fairfield Co.: Bethel, Brookfield Danbury, New Fairfield, Newtown Redding & Sherman; Litchfield Co.: Bridgewater, Kent, New Milford & Roxbury	10.00	.75	.75		

DECISION NO. CT78-3055

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
BRICKLAYERS (CONT'D):					
Litchfield Co.: Barkhamsted, Bethlehem, Canaan, Colebrook, Cornwall, Goshen, Harwinton, Litchfield, Morris, New Hartford, Norfolk, N. Canaan, Salisbury, Sharon, Torrington, Warren, Washington, Winchester	9.80	.75	.75		
Windham Co.	10.20	.75	.75		
Litchfield Co.: Plymouth, Thomaston, Watertown, & Woodbury	9.95	.75	.75		
BRICKLAYERS (Heavy & Highway Construction):					
Except Towns of Darien, Greenwich & Stamford	9.50	.75	.75	b	
Darien & Stamford	9.83	.83	.25	b	
Greenwich	9.90	.55	.55	b	
CARPENTERS; Millwrights; Pile-drivermen & Soft floor layers (Building Construction):					
Fairfield Co.: Greenwich	10.10	.85	.65		.05
Fairfield Co.: Bridgeport, Easton, Fairfield, Monroe, Shelton, Stratford, Trumbull, Weston, & Westport	10.25	.90	.65	c	a
Fairfield Co.: Bethel, Brookfield, Danbury, Darien, New Canaan, New Fairfield, Newtown, Norwalk, Redding, Ridgefield, Sherman, Stamford & Wilton; Litchfield Co.: Barkhamsted, Bethlehem, Bridgewater, Canaan, Colebrook Cornwall, Goshen, Kent, Litchfield, Morris, New Hartford, New Milford, Norfolk, North Canaan, Roxbury, Salisbury, Sharon, Torrington, Warren, Washington, Winchester & Woodbury					
Commercial	10.10	.90	.65		.10
Residential	6.90	.90	.65		.10

DECISION NO. CT78-3055

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
Litchfield Co.: Harwinton, Plymouth, Thomaston & Watertown	10.10	.80	.55		.05
Windham Co.	10.25	.90	.65		.03
CARPENTERS (Heavy & Highway Construction):					
Fairfield Co.: Bridgeport, Easton, Fairfield, Monroe, Shelton Stratford, Trumbull, Weston & Westport	10.05	.90	.65	f	e
Fairfield Co.: Bethel, Brookfield, Danbury, Darien, New Canaan, New Fairfield, Newtown, Norwalk, Wilton, Redding, Ridgefield, Sherman, Stamford & Litchfield Co.: Remainder of Co.	10.05	.90	.65	f	.10
Fairfield Co.: Greenwich	10.10	.90	.65	f	.02
Litchfield Co.: Harwinton, Plymouth, Thomaston & Watertown	10.05	.90	.65		.05
Windham Co.:	10.05	.90	.65	f	.03
ELECTRICIANS:					
Fairfield Co.: Norwalk (E. of Five Mile River), Weston, Westport & Weston Building Construction	10.50	.68	3%+.30	6%	½%
Fairfield Co.: Bethel, Bridgeport, Brookfield, Danbury, Easton, Fairfield, Monroe, New Fairfield, Newton, Redding Ridgefield, Shelton, Sherman, Stratford & Trumbull; Litchfield Co.: Bridgewater & New Milford Building construction	11.05	1.10	3%+.30		½%
Residential construction	5.65	1.10	3%+.20		½%
Fairfield Co.: Darien, Greenwich, New Canaan & Stamford	12.00	6%+e	9½%	10%	.06
Litchfield Co.: Plymouth Building Construction	10.17	1.00	3%+.50		½%
Residential construction	5.53	1.00	3%+.50		½%
Windham Co.: Building construction	11.30	1.50	3%+.50		½%
Litchfield Co.: Rem. of Co.	10.40	1.22	3%.60	g	1/8%

DECISION NO. CT78-3055	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
ELEVATOR CONSTRUCTORS	10.35	.545	.35	4%+h+i	.02
ELEVATOR CONSTRUCTORS' HELPERS	7.245	.545	.35	4%+h+i	.02
ELEVATOR CONSTRUCTORS' HELPERS (PROB.)	5.175				
GLAZIERS:					
Fairfield Co.: Greenwich	9.10	.66	1.66	.365	.03
Fairfield Co.: Rem of Co.; & Litchfield Co.	9.83	.95	.47	j	.01
Windham Co.	11.41	.84	.55	k	
IRONWORKERS:					
Ornamental; Reinforcing; Structural; and Precast concrete erection	12.25	.75	1.45	l	.08
LATHERS:					
Fairfield Co.: Bridgeport, Easton, Fairfield, Monroe, Redding, Ridgefield, Shelton, Stratford, Trumbull, Weston, Westport & Wilton	10.00	.50	.40		.01
Fairfield Co.: Greenwich, New Canaan Norwalk & Stamford	8.51	.35	.20	.7>	.01
Fairfield Co.: Bethel, Brookfield, Danbury, New Fairfield Newton & Shetman; Litchfield Co.: Bethlehem, Bridgewater, Cornwall, Goshen, Harwinton, Litchfield, Morris, New Milford N. Canaan, Plymouth, Roxbury, Thomaston, Torrington Warren, Washington, Watertown & Woodbury	9.65	.35	.30		.01
Litchfield Co.: Barkhamsted, Colebrook, New Hartford, Norfolk & Winchester; Windham Co.: Chaplin, Hampton, Scotland & Windham	9.90	.50	.30		.01
Windham Co.: Danielson	9.25	.45	.55		.01
LEADBURNERS	9.25	.35		m	.01
LINE CONSTRUCTION:					
Fairfield Co.: Darien, Greenwich, New Canaan, Stamford & that portion of Norwalk, w. of Five Mile River					

DECISION NO. CT78-3055	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
Linemen	9.33	3-3/4%	6%+n+o	10%	4%
Cable splicers	9.33	3-3/4%	6%+n+o	10%	4%
Driver groundmen	9.33	3-3/4%	6%+n+o	10%	4%
Fairfield Co.: Rem. of Co.; Litchfield Co.; & Windham Co.					
Linemen	10.92	.70	3%+.50	p	
Equipment operators	10.19	.70	3%+.50	p	
Driver groundmen	7.87	.70	3%+.50	p	
MARBLE SETTERS' HELPERS:					
Fairfield Co.: Darien, Greenwich, Norfolk, Stamford & Westport	7.33	.74	.88	q	
MARBLE SETTERS' HELPERS; Terrazzo workers' helpers; & Tile setters' helpers:					
Fairfield Co.: Rem. of Co.; Litchfield Co.; & Windham Co.	8.65	.50	.65		
PAINTERS:					
Bridge:					
Structural steel	12.00				
Spray	15.50				
Sandblasting or power tools	13.00				
Fairfield Co.: Greenwich					
Brush; Structural steel; paperhangers; Tapers	8.40	.20	.75	.30	
Spray	12.60	.20	.75	.30	
Fairfield Co.: Bridgeport, Easton, Fairfield, Southport, Stratford and Trumbull					
Brush	8.65	.50	.65	r	
Spray	10.65	.50	.65	r	
Taper	9.04	.50	.65	r	
Fairfield Co.: Darien, Stamford, New Canaan, Norwalk, Weston, Westport & Wilton					
Brush	8.35	.50	.50		.01
Roller, paperhangers, tapers epoxy	8.85	.50	.50		.01
Fairfield Co.: Bethel, Brookfield, Danbury, New Fairfield, Newton, Redding, Ridgefield, Sandy Hook & Sherman; Litchfield Co.: New Milford					

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DECISION NO. CT78-3055	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
Brush, Roller	10.85	.50	.50	r	
Steel	11.95	.50	.50	r	
Paperhangers; Tapers	10.85	.50	.50	r	
Epoxy	12.35	.50	.50	r	
Residential	9.10	.50	.50	r	
Fairfield Co.: Byram					
Brush	8.15	.45	45+.426/7	.40	
Spray	9.15	.45	45+.426/7	.40	
Steel & Swing stage & boatswain Chair	9.313/7	.45	45+.426/7	.40	
Drywall taper	8.95	.45	45+.426/7	.40	
Fairfield Co.: Monroe & Shelton					
Brush	10.75	.50	.80	s	.01
Hand roller; Paperhangers	11.25	.50	.80	s	.01
Structural steel, epoxy, polyester	11.75	.50	.80	s	.01
Spray	14.75	.50	.80	s	.01
Windham Co.: Willimantic & Windham					
Brush; Tapers	10.00	.80	.60		
Paperhangers	10.50	.80	.60		
Riding steel; steamcleaning; sandblasting; tanks; towers; & Hazardous work	10.58	.80	.60		
Spray	13.00	.80	.60		
Litchfield Co.: Bantam, Barkhamsted, Canaan, Colebrook, Cornwall, Goshen, Harwinton, Kent, Litchfield, New Hartford, New field, N. Canaan, Plymouth, Salisbury, Sharon, Terryville, Torrington, Warren, Winchester & Winsted					
Brush; Roller; Taping	9.60	.80	.45		
Spray; High	12.00	.80	.45		
Epoxy	9.85	.80	.45		
Paperhanging	10.10	.80	.45		
Work over 60' & Boatswain chair; Open steel	14.40	.80	.45		
Swing Stage 40-60	10.60	.80	.45		

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DECISION NO. CT78-3055	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
Windham Co., except Willimantic & Windham					
Brush	9.90	.50	.70		.01
Paperhangers	10.20	.50	.70		.01
Sign	10.28	.50	.70		.01
Taping	10.30	.50	.70		.01
Roller	10.40	.50	.70		.01
Structural Steel	10.70	.50	.70		.01
Spraying oil paint	13.05	.50	.70		.01
Spraying epoxy	15.58	.50	.70		.01
PLUMBERS:					
Windham Co.: Windham	10.10	.62	.62		.20
PLUMBERS; Steamfitters:					
Fairfield Co.: Greenwich	10.00	.45	.70		.02
Fairfield Co.: Bridgeport, Easton, Fairfield, Monroe, Shelton, Stratford & Trumbull	10.49	.75	.70	.42	.01
Fairfield Co.: Georgetown, Norwalk, S. Norwalk, Weston, Westport & Wilton	9.50	.75	.50	.36	.02
Fairfield Co.: Bethel, Brookfield, Danbury, New Fairfield, Newton, Redding, Ridgefield & Sherman; Litchfield Co.: Bridgewater & New Milford	10.70	.75	.70	.50	.02
Fairfield Co.: Darien, New Canaan & Stamford	10.55	.75	.70	4%	.02
Litchfield Co.: Bantam, Barkhamsted, Canaan, Colebrook, Cornwall, Falls Village, Goshen, Harwinton, Kent, Lakeville, Litchfield, Morris, New Hartford, Norfolk, N. Canaan, Salisbury, Sharon, Torrington, Warren, Winchester & Winstead	9.64	.83	.70	t	.05
Litchfield Co.: Bethlehem, New Preston Plymouth (incl. Terryville), Roxbury, Thomaston, Washington, Watertown & Woodsbury	10.00	.83	.70	t	.05
Windham Co., except Windham	11.92	.60	.70	u	.05

DECISION NO. CT78-3055

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
ROOFERS:					
Fairfield Co., except that portion of Fairfield Co. bounded on the e. by the eastern boundary of Greenwich; Litchfield Co.: Bethlehem, Bridgewater, Kent New Milford, Roxbury, Washington & Woodbury	10.90	1.05	.45		
Litchfield Co.: Bantam, Canaan, Colebrook, Cornwall, Cornwall Bridge, E. Canaan, Falls Village, Gaylordsville, Goshen Lakeside, Litchfield, Marbledale, Morris, New Hartford, New Preston, Norfolk, Northfield, Oakville, Prequabuck, Pine Meadow Pleasant Valley, Plymouth, Riverton, Salisbury, Sharon, S. Kent, Taconic, Terryville, Thomaston, Torrington, Washington Depot, Watertown, W. Cornwall, W. Goshen, Winchester Center & Winsted; Windham Co.					
Composition	8.75	.525	.70	.55	
Slate & tile	9.25	.525	.70	.55	
Fairfield Co.: that portion of Fairfield Co. bounded on the e. by the eastern boundary of Greenwich					
Slate & Tile	9.20	.50	1.40	1.40	
Helpers	7.50	.50	1.00	1.00	
SHEET METAL WORKER:					
Fairfield Co.; Litchfield Co.	11.50	1.28	1.04		.02
Windham Co.	10.55	.70	.86		.05
SPRINKLER FITTERS	11.75	.75	1.05		.08
STEAMFITTERS:					
Windham Co.: Windham	10.27	1.16	.66		.13
TILE SETTERS' HELPERS:					
Fairfield Co.: Darien, Greenwich, Stamford, Norfolk & Westport	7.58	.50	1.27		

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PAID HOLIDAYS:
A-New Year's Day; B-Memorial Day; C-Independence Day; D-Labor Day; E-Thanksgiving Day; F-Christmas.

FOOTNOTES:

a. 7 paid holidays: A, C, D, E, F, Decoration Day & Good Friday.
b. 1 paid holiday: Good Friday. Employee must work 3 days during the work week in which the holiday falls, if scheduled, and if scheduled, the working day before and the working day after the holiday.
c. 4 paid holiday: B, C. D, and Good Friday. Employee must be employed 14 consecutive days immediately, prior to the holiday.
d. 3 paid holidays: C, D and E.
e. $7.00 per day
f. 3 paid holidays: B, C, and D.
g. The last 4 regular working hours prior to Christmas shall be paid half day.
h. 6 paid holidays: A through F
i. Employer contributes 4% of basic hourly rate for 5 years or more of service or 2% basic hourly rate for 6 months to 5 years of service as vacation pay credit.
j. 9 paid holidays: A through F, Washington's Birthday, Good Friday and Columbus Day.
k. 9 paid holidays: A through F, Washington's Birthday, Good Friday and Columbus Day.
l. The last 4 hours on Christmas Eve is a pid half day if employee has worked 5 consecutive days prior to Christmas Eve.
m. 9 paid holidays: A through F, Washington's Birthday, Good Friday, and Christmas Eve provided the employee has worked 45 full days for the employer during the 120 days prior to the holiday and is availabel for work the day preceding and following the holiday.
n. 1% of the gross electrical labor payroll.
o. Employer contributes $1.50 per day to a supplemental unemployment fund.
p. 9 paid holidays: A through F, Washington's Birthday, Good Friday, and a floating holiday per year provided the employee has been employed for a period of 5 working days prior to the holidays and works the scheduled work days immediately preceding and following the holidays.
q. 1 paid holiday: St. Patrick's Day.
r. 2 paid holidays: C and D providing the employee works the day before and the day after the holiday.
s. 4 paid holidays: B, C, D and E providing the employee works the day before and the after the holiday.
t. 2 paid holidays: B and D and half day paid holiday the Friday after Thanksgiving and the last working day before Christmas and Good Friday paid half day.
u. 1 paid holiday: D
y. 3% of gross earnings to SASMI

DECISION NO. CT78-3055

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
POWER EQUIPMENT OPERATORS (Building Construction)					
Derrick; Hoisting engineer 2 drums and over; Hoisting structural steel; Pile driver & Setting Stone	11.55	.90	.85	a	.10
Dragline; Forklift - over 4' lift; Front end loader - 7cy. or over; Gradall; Hoisting engineer (all types of equipment where a drum and cable are used to hoist, pull, motive power or operation); Koehring scooper loader and/or hoe; master mechanic; shovel; & tower crane	11.43	.90	.85	a	.10
Maintenance engineer	11.33	.90	.85	a	.10
Central mix operator; Coleman loader and screeing plant or similar equipment; combination hoe and loader over ½ yd; Conveyors - regardless of motive power; Front end loader 3cy. up to 7 cy.; Joy drill limited to joy heavy weight champion or equivalent; Mucking machine; Post hole digger; pumporete machine; rock boring machine; vibratory hammer; welder & well digger	11.07	.90	.85	a	.10
Compressor battery operator	10.26	.90	.85	a	.10
Asphalt spreader	10.85	.90	.85	a	.10
Bulldozer; Carry-all operators; Grader; & Scraper pan	10.79	.90	.85	a	.10
Combination hoe and loader machine; concrete mixer - 5 bags over; Front end loader under 3 cy.; power-stone spreader	10.74	.90	.85	a	.10

DECISION NO. CT78-3055

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
Air and steam valve	9.99	.90	.85	a	.10
Compressor; generator; pump and well point; welding machine	9.88	.90	.85	a	.10
Fork lift not over 4'; & Steam Jenny	10.63	.90	.85	a	.10
Mechanical heater	9.71	.90	.85	a	.10
Roller	10.52	.90	.85	a	.10
Dinky machine; power pavement breaker	10.36	.90	.85	a	.10
Fireman (High pressure	9.50	.90	.85	a	.10
Oiler	9.02	.90	.85	a	.10
Crane with boom, excluding jib, over 150' - $.25 extra					
Crane with boom, excluding jib, over 200' - $.50 extra					
LABORERS (Heavy and Highway Construction):					
Laborers	7.95	.50	.70		.10
Acetylene burners; Asphalt raker; Chain saw operator; Concrete & power buggy operators; Concrete saw operator; fence & guard rail erectors; form setters; hand operated concrete vibrator operators; hand operated vibratory compactor operators; Mason tenders; pipelayers; pneumatic drill operators; pneumatic gas & electric drill operators; powdermen & wagon drill operators	8.20	.50	.70		.10
Air track operators; block paver; curb setters	8.45	.50	.70		.10
Blasters	8.70	.50	.70		.10

DECISION NO. CT78-3055

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
LABORERS (Building Construction)					
Laborer	7.95	.50	.70		.10
Asphalt rakers, concrete & power buggy ops, concrete saw ops, chain saw ops, fence & guard rail erectors, form setters, pipelayers, dry stone wall builders, mason tenders, pneumatic drill ops, pneumatic gas & electric drill ops, powdermen & wagon drill operators	8.20	.50	.70		.10
Air track ops, block pavers; and curb setters	8.45	.50	.70		.10
Blasters	8.70	.50	.70		.10
Open air caisson, cylindrical work and boring crew:					
Top man	7.95	.50	.70		.10
Bottom man	8.45	.50	.70		.10

DECISION NO. CT78-3055

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
POWER EQUIPMENT OPERATORS:					
CLASS 1	11.58	.90	.85	a	.10
CLASS 2	11.42	.90	.85	a	.10
CLASS 3	11.08	.90	.85	a	.10
CLASS 4	10.88	.90	.85	a	.10
CLASS 5	10.73	.90	.85	a	.10
CLASS 6	10.53	.90	.85	a	.10
CLASS 7	10.32	.90	.85	a	.10
CLASS 8	9.41	.90	.85	a	.10
CLASS 9	9.52	.90	.85	a	.10
CLASS 10	9.89	.90	.85	a	.10
CLASS 11	10.24	.90	.85	a	.10
CLASS 12	9.05	.90	.85	a	.10
CLASS 13	9.82	.90	.85	a	.10
Crane with 150' boom - $.25 extra					
Crane with 200' boom - $.50 extra					

CLASSIFFICATIONS: POWER EQUIPMENT OPERATORS

CLASS 1: Erecting and handling structural steel; front end loader (7cy. or over)
CLASS 2: Piledriver; Power shovel and crane; Dragline; Gradall; Trenching machine; lighter derrick; paver (concrete); derrick (stiff leg and guy); steel pile sheeting; koehring loader (skooper) master mechanic
CLASS 3: Drill (joy heavy weight champion or equivalent); side boom; loader (euclid); mucking machine; pumpcrete; rock and earth boring machine; Post hole digger; well digger; & hammer (vibratory); central mix; combination hoe & loader (over ½ yd)
CLASS 4: Asphalt spreader
CLASS 5: Front end loader (3yds. or over); Grader; power stone spreader; combination hoe and loader
CLASS 6: Asphalt roller; bulldozer; carryall; maintenance engineer; concrete mixer (5 bags and over); Welder
CLASS 7: Front end loader (under 3yds.); roller; pOwer chipper; fork lift; finishing machine; asphalt plant; power pavement breaker; dinky machine
CLASS 8: Compressor; pump
CLASS 9: Fireman (high pressure)
CLASS 10: Well point system
CLASS 11: Compressor battery
CLASS 12: Oiler
CLASS 13: Batch plant; bulk cement plant

DECISION NO. CT78-3055

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
TRUCK DRIVERS (Building, Heavy and Highway Construction)					
CLASS 1	7.81	.74	.725	a	
CLASS 2	7.91	.74	.725	a	
CLASS 3	8.01	.74	.725	a	
CLASS 4	7.96	.74	.725	a	
CLASS 5	8.06	.74	.725	a	
CLASS 6	8.11	.74	.725	a	

CLASSIFICATIONS: TRUCK DRIVERS

CLASS 1: Two axle trucks; helpers
CLASS 2: Three axle trucks; two axle ready mix
CLASS 3: Four axle trucks; heavy duty trailer-up to 40 tons
CLASS 4: Three axle ready-mix
CLASS 5: Four axle ready-mix; specialized earth moving equipment other than conventional type on-the-road trucks and simi-trailer (including Euclids)
CLASS 6: Heavy duty trailer-40 tons and over

PAID HOLIDAYS:
A-New Year's Day; B-Memorial Day; C-Independence Day; D-Labor Day; E-Thanksgiving Day & F-Christmas Day

FOOTNOTE:
a. 7 paid holidays: A through F and Good Friday

SUPERSEDEAS DECISION

STATE: CONNECTICUT COUNTIES: *SEE BELOW
DECISION NUMBER: CT78-3056 DATE: DATE OF PUBLICATION
Supersedes Decision No. CT78-3004 dated February 17, 1978 in 43 FR 7117
DESCRIPTION OF WORK: Building Construction (excluding single family homes and garden type apartments up to and including 4 stories), heavy (excluding tunnel construction) and highway construction:

*Hartford, Middlesex, New Haven *New London, and Tolland Counties	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
ASBESTOS WORKERS: Hartford Co.: Avon, Berlin, Bloomfield, Bristol, Burlington, Canton, E. Hartford, E. Windsor, Farmington, Glastonbury, Hartford, Manchester, Marlborough, New Britain, Newington, Plainville, Rocky Hill, Simsbury, Southington, S. Windsor, W. Hartford, Wethersfield & Windsor; Middlesex Co.; New Haven Co.; New London Co.: Bozrah, Colchester, E. Lyme, Franklin, Groton, Lebanon, Lyme, Montville, New London, Norwich, Old Lyme, Salem, Sprague & Waterford; & Tolland Co.: Andover, Bolton, Columbia, Conventry, Ellington, Hebron, Mansfield, Tolland, Vernon & Willington	$11.84	.75	1.16		
Hartford Co.: E. Granby, Enfield, Granby, Hartland, Suffield & Windsor Locks; Tolland Co.: Somers, Stafford & Union	10.65	.94	1.25		.01
New London Co.: Griswold, Ledyard, Lisbon, N. Stonington, Preston, Stonington & Voluntown	10.28	1.00	1.18		
BOILERMAKERS	10.00	.60	10%		.01
BRICKLAYERS:; Cement masons; Finishers; Marble masons; Plasterers; Stonemasons; Terrazzo workers & Tile setters (Building Construction): Hartford Co.: Avon, Bloomfield, Burlington, Enfield, E. Granby, E. Hartford, Farmington, Glastonbury, Granby, Hartford, Hartland, Manchester, Marlborough, Rocky Hill, Simsbury					

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	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
BRICKLAYERS (CONT'D): S. Windsor, Suffield, Thompsonville, W. Hartford, Wethersfield, Windsor, Windsor Locks; Tolland Co.: Andover, Bolton, Columbia Coventry, Ellington, Hebron, Mansfield, Somers, Stafford, Storrs, Tolland, Union, Vernon & Willington	$ 9.75	.90	.75		
Hartford Co.: Berlin, New Britain, Newington, & Southington; New Haven Co.: Meriden, Wallingford, Cheshire (North of Route 68)	10.05	.75	.75		
Hartford Co.: Canton	9.80	.75	.75		
Middlesex Co.: & New London Co.	10.20	.75	.75		
New Haven Co.: Bethany, Branford, E. Haven, Guilford, Hamden, Madison, New Haven, N. Brandford, N. Haven, Orange, W. Haven, Woodbridge, Rem. of Milford & Chesire	10.10	.75	.75		
New Haven Co.: Ansonia, Derby, Oxford, Seymour & Southbury	9.70	.75	.75		
Hartford Co.: Bristol,& Plainville; New Haven Co.: Beacon Falls, Middlebury, Mixville, Naugatuck, Prospect, Waterbury & Wolcott	9.95	.75	.75		
New Haven Co.: Milford (West of Indian River to the Orange Town line)	10.05	.75	.75		
BRICKLAYERS (Heavy & Highway Construction)	9.50	.75	.75	b	
CARPENTERS: MILLWRIGHTS; PILEDRIVERMEN: RESILIENT FLOOR LAYERS (Building Construction)					
Hartford Co.: Hartford, West Hartford, Avon, Farmington, Simsbury, Bloomfield, Windsor, East Granby, Granby, Windsor Locks, Suffield, Enfield, East Windsor, South Windsor, East Hartford, Manchester, Glastonbury, Rocky Hill, Wethersfield, Hartland; Tolland Co.: Stafford, Somers, Tolland, Ellington, Bolton Vernon	10.35	.90	.65		.05
Hartford Co.: New Britain, Newington, Berlin, Southington Plainville, Burlington, Canton, Bristol; New Haven Co.: Meriden, Wallingford, New Haven, East Haven, Brandord, Guilford, Madison, North Branford, North Haven, Hamden, West Haven, Orange (east of Orange Center Road and North of Route 1; also north of Route 1 and east of the Oyster River), Cheshire, Waterbury, Wolcott, Middlebury, Southbury, Naugatuck, Prospect, Bethany Beacon Falls, Woodbridge; Middlesex Co.	10.10	.80	.55		.05
New Haven Co.: Milford, Oxford, Derby, Seymour, Ansonia, Orange (that part west of Orange Center Rd. and south of Route 1, and that part south of Route 1 and West of the Oyster River	10.25	.90	.65	c	e
New London Co.: Tolland Co.: Mansfield, Union, Willington, Coventry, Hebron, Columbia, Andover	10.25	.90	.65		.03
CARPENTERS (Heavy & Highway Construction):					
New Haven Co.: Ansonia, Derby, Milford, Orange (W. of Orange Center Road & S. of Rte #1 & W. of the Oyster River), Oxford & Seymour	10.05	.90	.65	f	e
Hartford Co.; Middlesex Co.; & New Haven Co.: Remainder of Co.; Tolland Co.: Bolton, Ellington, Somers, Stafford, Tolland & Vernon	10.05	.90	.65	f	.05

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DECISION NO. CT78-3056	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
CARPENTERS CONT'D):					
New London Co.; Tolland Co.: Andover, Columbia, Conventry, Hebron, Mansfield, Union & Willington	10.05	.90	.65	f	.03
ELECTRICIANS:					
New Haven Co.: Milford	10.40	1.10	3%+.30		½%
Hartford Co.: Berlin, Bristol, New Britain, Newington, Plainville & Southington	10.17	1.00	3%+.30		½%
Hartford Co.: Suffield & Enfield (portion of Thompsonville West of George Washington Road and North of Hazard Ave)	9.85	.70	3%+.30	.43	.03
Hartford Co.: Hartland; New Haven Co.: Beacon Falls, Middlebury, Naugatuck, Oxford, Prospect, Seymour, Southbury, Waterbury & Wolcott	10.40	1.22	3%+.60	h	1/8%
Hartford Co.: Rem. of Co.; Middlesex Co.: Cromwell, Middlefield, Middletown & Portland; New London Co.: Bozrah Colchester, Franklin, Lisbon, Montville, N. Stonington, Norwich, Griswold, Lebanon, Ledyard, (except Submarine Base) Preston, Salem, Sprague, Stonington & Voluntown & Tolland Co.	11.30	1.50	3%+.50		½%
Middlesex Cc.: Remainder of County					
New Haven Co.: Reaminder of Co.; ford; New London Co.: E. Lyme, New London, Fishers Island Sound Old Lyme, Groton, Waterford & Ledyard (Submarine Base Only	10.36	1.00	3%+.40		¼ of 1%
ELEVATOR CONSTRUCTORS	10.35	.545	.35	3%+i+j	.02
ELEVATOR CONSTRUCTORS' HELPERS	7.245	.545	.35	3%+i+j	.02
ELEVATOR CONSTRUCTORS' HELPERS (PROB.)	5.175				

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DECISION No. CT78-3056	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
GLAZIERS:					
New Haven Co., except Wallingford	9.83	.95	.47	k	.01
Hartford Co.; Middlesex Co.; New Haven Co.; Wallingford; New London Co.; & Tolland Co.	11.81	.84	.55	l	
IRONWORKERS:					
Ornamental; Reinforcing; Structural and Precast concrete erection	12.25	.75	1.45	m	.08
LATHERS:					
Hartford Co.: Bristol, Southington; New Haven Co.: Beacon Falls, Bethany, Cheshire, Meriden, Middlebury, Naugatuck, Oxford, Prospect, Southbury, Waterbury & Wolcott	9.65	.35	.30		.01
Hartford Co.: Avon, Berlin, Bloomfield, Burlington, Canton, E. Granby, E. Hartford, Farmington, Glastonbury, Granby, Hartford, Manchester, Marlborough, New Britain, Newington, Plainville, Rocky Hill, Simsbury, S. Windsor, W. Hartford, Windsor & Windsor Locks; Middlesex Co.: Cromwell, E. Haddam, E. Hampton, Middlefield, Middletown & Portland; New London Co.: Bozrah, Colchester, E. Lyme, Franklin, Grofton, Lebanon, Lisbon, Lyme, Salem, Sprague & Waterford; Tolland Co.: Andover, Bolton, Columbia, Coventry, Hebron, Mansfield & Vernon	9.90	.50	.30		.01
Hartford Co.: Broad Brook, Enfield, Hazardville, Melrose, Suffield, Thompsonville & Warehouse Point; Tolland Co.: Crystal Lake, N. Somers, Somers, Straffold, Stafford Springs, Straffordville & Union	9.40	.45	.25		.01

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	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
LATHERS (CONT'D):					
Middlesex Co.: Chester, Clinton, Deep River, Durham, Essex, Haddam, Killingworth, Old Saybrook & Westbrook; New Haven Co.: Ansonia, Branford, Derby, E. Haven, Guilford, Hamden, Madison, Milford, New Haven, N. Branford, N. Haven, Orange, Seymour, Wallingford, W. Haven & Woodbridge	9.80	.75	.45		.01
LEADBURNERS	9.25	.35		n	.01
LINE CONSTRUCTION:					
Linemen	10.92	.70	3%+.50	o	
Equipment operators	10.19	.70	3%+.50	o	
Driver groundmen	7.87	.70	3%+.50	o	
MARBLE SETTERS' HELPERS; Terrazzo workers' helpers; & Tile setters' helpers	8.75	.50	.65		
PAINTERS:					
Bridge and Highway	12.45				
Structural steel	12.45				
Sandblastings or power tools	13.45				
Spray	15.95				
New Haven Co.: Milford (up to Gulf Street)					
Brush	8.65	.50	.65	p	
Taping	9.04	.50	.65	p	
Spray	10.65	.50	.65	p	
Hartford Co.: Avon, Bloomfield, Broad Brook, Canton, E. Granby, E. Hartford, E. WIndsor, Enfield, Farmington, Glastonbury, Granby, Hartford, Manchester, Marlborough, Rocky Hill, Poquonock, Simsbury, S. Windsor, Suffield, S. Manchester, W. Hartford, W. Simsbury, Wethersfield, Windsor, & Windsor Locks; Tolland Co.: Andover, Ashford, Bolton, Columbia, Conventry, Ellington, Herbron, Mansfield, Rockville, Strafford, Somers, Tolland,					

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	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
PAINTERS (CONT'D):					
Union, Vernon, & Willington					
Brush; Tapers	10.00	.80	.60		
Paperhangers	10.50	.80	.60		
Riding steel; steamcleaning; Sandblasting; Tank; Towers, & Hazardous work	10.58	.80	.60		
Spray	13.00	.80	.60		
Hartford Co.: Berlin, Bristol, Burlington, E. Berlin, Forestville, Hartland, Kensington, Milldale, New Britain, Newington, Plainville, Plantsville, Southington, & Unionville; New Haven Co.: Cheshire, Guildord Madison, Meriden, & Wallingford; Middlesex Co.: Chester, Clinton, Cromwell, Deep River, Durham, E. Haddam, E. Hampton, Essex, Haddam, Higganum, Ivoryton, Killingworth, Middlefield, Middletown, Moodus, Norfolk, Portland, Saybrook, & Westbrook					
Brush; Roller; Taping	9.60	.80	.45		
Spray	12.00	.80	.45		
Epoxy	9.85	.80	.45		
Paperhanging	10.10	.80	.45		
Work over 60' & Boatswain chair; Open Steel	14.40	.80	.45		
Swing stage 40 - 60'	10.60	.80	.45		
New Haven Co.: Ansonia, Beacon, Falls, Derby, Oxford, & Seymour					
Brush	10.75	.50	.80	q	.01
Hand roller; Paperhangers	11.25	.50	.80	q	.01
Structural steel, epoxy polyester	11.75	.50	.80	q	.01
Spray	14.75	.50	.80	q	.01
New Haven Co.: Bethany, Branford, E. Haven Hamden, Milford, (s. on Gulf Street & n. on North Street, New Haven, N. Bradford, N. Haven, Orange, W. Haven, Woodbridge, & Woodmont					

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DECISION NO. CT78-3056	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
PAINTERS (CONT'D)					
Brush; Sandblasting	9.45	.50	.60		.02
Tapers	9.80	.50	.60		.02
Paperhangers	9.95	.50	.60		.02
Spray	12.10	.50	.60		.02
Structural steel	9.70	.50	.60		.02
New Haven Co.: Middlebury, Naugatuck, Prospect, Roxbury, Southbury, Thomaston, Washington, Waterbury, Watertown, Wolcott, & Woodbury					
Brush	8.80	.50	.60		
Paperhanger & Tapers	9.05	.50	.60		
Spray	13.50	.50	.60		
New London Co.: Norwich					
Brush	9.90	.50	.70		.01
Paperhangers	10.70	.50	.70		.01
Sign	10.28	.50	.70		.01
Taping	10.30	.50	.70		.01
Roller	10.40	.50	.70		.01
Structural steel	10.70	.50	.70		.01
Spraying oil paint	13.05	.50	.70		.01
Spraying epoxy	15.58	.50	.70		.01
PLUMBERS:					
Hartford Co.: Avon, Bloomfield, Burlington, Canton, E. Granby, E. Hartford, E. Windsor, Enfield, Farmington, Glastonbury, Granby, Hartford, Manchester, Marlborough Newington, Rocky Hill, Simsbury, S. Windsor, Suffield, W. Hartford, Wethersfield, Windsor, Windsor, Locks Middlesex Co.: Chester, Cromwell, Deep River, E. Haddam, E. Hampton, Haddam, Maromas (Atomic River Project) Middlefield, Middletown & Portland; Tolland Co.: Andover, Bolton, Columbia, Coventry, Ellington, Hebron, Mansfield, Somers, Stafford, Storrs, Tolland, Union, Vernon, &					

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DECISION NO. CT78-3056	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
Willington	10.31	1.10	1.39		.10
PLUMBERS: Steamfitters:					
New Haven Co.: Milford	10.49	.75	.70	.42	.01
Hartford Co.: Southington; Middlesex Co.: Durham; New Haven Co.: Cheshire, Meriden & Wallingford	10.35	.75	.70	r	.03
Hartford Co.: Berlin, Bristol, E. Berlin, Kensington, New Britain, & Plainville	10.34	.75	.70	s	.03
Middlesex Co.: Clinton, Killingworth, S Westbrook; New Haven Co.: Branford, Derby, E. Haven, Orange, W. Haven, & Woodbridge	10.55	.75	.70	t	.02
Middlesex Co.: Essex, Ivoryton,, Old Saybrook, & Saybrook; New London Co.: Bozrah, Colchester, E. Lyme, Montville, New London, N. Stonington, Norwich, Old Lyme, Preston, Salem, Sprague, Stonington, Voluntown, & Waterford	11.92	.60	.70	u	.05
New Haven Co.: Ansonia, Beacon Falls, Bethany, Naugatuck, Oxford, Prospect, & Seymour					
New Haven Co.: Middlebury, Southbury, S. Britain, Waterbury & Wolcott					
Hartford Co.: Hartland	10.45	.88	.70	v	.05
ROOFERS:					
New Haven Co.: Ansonia, Beacon Falls, Bethany, Branford, Derby E. Haven, Guilford, Hamden, Madison, Milford, Middlebury, Naugatuck, New Haven, N. Branford, N. Haven, Orange, Oxford, Seymour, Union City, W. Haven, & Woodbridge	10.15	1.05	.45		
Hartford Co.; Middlesex Co.; New Haven Co.: Cheshire, Meriden, Prospect, Wallingford, & Wolcott; New London Co.; & Tolland Co.					
Composition	8.75	.525	.70	.55	
Slate, Tile	9.25	.525	.70	.55	

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	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
SHEET METAL WORKERS	10.55	.70	.86		.05
SPRINKLER FITTERS	11.75	.75	1.05		.08
STEAMFITTERS:					
Hartford Co.: Avon, Bloomfield, Burlington, Canton, E. Granby, E. Hartford, E. Windsor, Enfield, Farmington, Glastonbury, Granby, Hartford, Manchester, Marlborough, Newington, Rocky Hill, Simsbury, S. Windsor, Suffield, W. Hartford, Wethersfield, Windsor, & Windsor Locks; Middlesex Co.: Chester, Cromwell, Deep River, E. Haddam, E. Hampton, Haddam, Maromas (Atomic River Project), Middlefield, Middletown, & Portland; Tolland Co.: Andover, Bolton, Columbia, Conventry, Ellington, Hebron, Mansfield, Somers, Stafford, Storrs, Tolland, Union, Vernon, & Willington	10.57	1.36	.81		.13

DECISION NO. CT78-3056

PAID HOLIDAYS:
A-New Year's Day; B-Memorial Day; C-Independence Day; D-Labor Day; E-Thanksgiving Day; F-Christmas Day.

FOOTNOTES:

a. 7 paid holidays: A through F, and Good Friday.
b. 1 paid holiday: Good Friday. Employee must work 3 days during the work week in which the holiday falls, if scheduled, and if scheduled, the working day before and the working day after the holiday.
c. 4 paid holidays: B, C. D, and Good Friday. Employee must be employed 14 consecutive days immediately prior to the holiday.
d. 3 paid holidays: C, D, and E
e. $.50 per worker per year
f. 3 paid holidays: B, C, and D
h. The last 4 regular working hours prior to Christmas Day shall be paid half day.
i. 6 paid holidays: A through F
j. Employer contributes 4% of basic hourly rate for 5 years or more of service or 2% basic hourly rate for 6 months to 5 years of service as vacation pay credit.
k. 9 paid holidays: A through F, Washington's Birthday, Good Friday, and Columbus Day
l. 9 paid holidays: A through F, Washington's Birthday, Good Friday and Columbus Day
m. The last 4 hours on CHristmas Eve is a paid half day if employee has worked 5 consecutive days prior to CHristmas Eve
n. 9 paid holidays: A through F, Washington's Birthday, Good Friday and Xmas Eve provided the employee has worked 45 full days for the employer during the 120 days prior to the holidays and works the scheduled work days immediately preceding and following the holidays
o. 9 paid holidays: A through F Washington's Birthday, Good Friday and a floating holiday pre year provided the employee has been employed for a period of 5 working days prior to the holidays and works the scheduled work days immediately preceding and following the holidays
p. 2 paid holidays: C and D providing the employee works the day before and the day after the holiday
q. 4 paid holidays: B, C, D, and E providing the employee works the day before and the dat after the holiday
r. 1 paid holiday: D
s. Paid holidays: D, and the Friday after Thanksgiving is a paid half day, and December 24, provided it falls on a working day, is a paid half day
t. 3½ paid holidays: C, D, E, and the half day Friday after Thanksgiving
u. 1 paid holidays: D
v. 2 paid holidays: B, D & half day paid holiday the Friday after Thanksgiving and the last working day before CHristmas, & Good Friday paid half day

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DECISION NO. CT78-3056

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
POWER EQUIPMENT OPERATORS: (BUILDING CONSTRUCTION)					
Derrick; Hoisting engineer 2 drums and over; Hoisting structural steel; Pile driver; & Setting stone	11.55	.90	.85	a	.10
Dragline; Fork lift - over 4' lift; Front end loader - 7 cy. or over; Gradall; Hoisting engineer (all types of equipment where a drum and cable are used to hoist, pull, or drag material regardless of motive power or operation); Koehring scooper loader and/or hoe; master mechanic; shovel; & tower crane	11.43	.90	.85	a	.10
Maintenance engineer	11.33	.90	.85	a	.10
Central mix operator; Coleman loader and screening plant or similar equipment; combination hoe and loader over ¼ yd.; Conveyors - regardless of motive power; Front end loader - 3 cy. up to 7 cy.; High pressure portable boiler; Joy drill - limited to joy heavy weight champion or equivalent; mucking machines; post hoe digger; pumpcrete machine; rock boring machine; vibratory hammer; welder; & Well digger	11.07	.90	.85	a	.10
Compressor battery operator	10.26	.90	.85	a	.10
Asphalt spreader	10.85	.90	.85	a	.10
Bulldozer; Carry-all operators; Grader; & Scraper pan	10.79	.90	.85	a	.10
Combinationhoe and loader machine; concrete mixer - 5 bags or over; front end loader under 3 cy.; powerstone spreader	10.74	.90	.85	a	.10

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DECISION NO. CT78-3056

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
POWER EQUIPMENT OPERATORS (CONT'D)					
Air and steam valve	9.99	.90	.85	a	.10
Compressor; generator; pump and well point; welding machine	9.88	.90	.85	a	.10
Fork lidt not over 4'; & Steam Jenny	10.63	.90	.85	a	.10
Mechanical heater	9.71	.90	.85	a	.10
Roller	10.52	.90	.85	a	.10
Dinky machine; Power pavement breaker	10.36	.90	.85	a	.10
Fireman (High pressure)	9.50	.90	.85	a	.10
Oiler	9.02	.90	.85	a	.10
Crane with boom, excluding jib, over 150' - $.25 extra					
Crane with boom, excluding jib, over 200' - $.50 extra					
LABORERS(Heavy and Highway Construction):					
Laborers	7.95	.50	.70		.10
Acetylene burners; Asphalt raker; chain saw operator; concrete & power buggy operators; Concrete saw operator; fence & guard rail erectors; Form setters; Hand operated vibratory compactor operators; Mason tenders; pipelayers; pneumatic gas & electric drill operators; powdermen & wagon drill operators	8.20	.50	.70		.10
Air track operator; block pavers; curb setters	8.45	.50	.70		.10
Blasters	8.70	.50	.70		.10

DECISION NO. CT78-3056

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
LABORERS (Building Construction)					
Laborers	7.95	.50	.70		.10
Asphalt rakers, concrete & power buggyops, concrete saw ops, chain saw ops, fence & guard rail erectors, form setters, pipelayers, dry stone wall builders, mason tenders, pneumatic gas & electric drill ops. powdermen, & wagon drill ops	8.20	.50	.70		.10
Air track ops, block pavers, and curb setters	8.45	.50	.70		.10
Blasters	8.70	.50	.70		.10
Open Air Caisson, Cylindrical Work and Boring Crew:					
Top man	7.95	.50	.70		.10
Bottom man	9.45	.50	.70		.10

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	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
POWER EQUIPMENT OPERATORS: (HEAVY & HIGHWAY CONSTRUCTION)					
Class 1	11.58	.90	.85	a	.10
Class 2	11.42	.90	.85	a	.10
Class 3	11.08	.90	.85	a	.10
Class 4	10.88	.90	.85	a	.10
Class 5	10.73	.90	.85	a	.10
Class 6	10.53	.90	.85	a	.10
Class 7	10.32	.90	.85	a	.10
Class 8	9.41	.90	.85	a	.10
Class 9	9.52	.90	.85	a	.10
Class 10	9.89	.90	.85	a	.10
Class 11	10.24	.90	.85	a	.10
Class 12	9.05	.90	.85	a	.10
Class 13	9.82	.90	.85	a	.10
Crane with 150' boom - $.25 extra					
Crane with 200' boom - $.50 extra					

POWER EQUIPMENT OPERATORS CLASSIFICATIONS

Class 1: Erecting and handling structural steel; front end loader (7 cy. or over)
Class 2: Piledriver; Power shovel and crane; dragline; gradline; gradall; trehcning machine; lighter derrick; paver (concrete); derrick (stiff leg and guy); Steel pile sheeting; koehring loader (skooper); master mechanic
Class 3: Drill (Joy heavy weight champion or equivalent); Side boom; loader (Euclid); Mucking machine; Pumpcrete; ROck and earth boring machine; post hole digger; well digger; & hammer (vibratory); central mix; combination hole & loader (over ½ yd)
Class 4: Asphalt
Class 5: Front end loader (3 yds. or over); grader; power stone spreader; combination hoe and loader
Class 6: Asphalt roller; bulldozer; carryall; maintenance engineer; concrete mixer (5 bags and over); welder
Class 7: Front end loader (under 3 yds.); roller; power chipper; fork lift; finishing machine; asphalt plant; power pavement breaker; dinky machine
Class 8: Compressor; pump
Class 9: Fireman (high pressure)
Class 10: WEll point system
Class 11: Compressor battery
Class 12: Oiler
Class 13: Batch plant; Bulk cement plant

DECISION NO. CT78-3056

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
TRUCK DRIVERS (Building, Heavy & Highway Construction)					
TRUCK DRIVERS:					
Class 1	7.81	.74	.725	a	
Class 2	7.91	.74	.725	a	
Class 3	8.01	.74	.725	a	
Class 4	7.96	.74	.725	a	
Class 5	8.06	.74	.725	a	
Class 6	8.11	.74	.725	a	

TRUCK DRIVERS

Class 1: Two axle trucks; helpers
Class 2: THree axle trucks; two axle ready-mix
Class 3: Four axle trucks; heavy duty trailer-up to 40 tons
Class 4: Three axle ready mix
Class 5: Four axle ready-mix; specialized earth moving equipment other than conventional type on-the-road trucks and semi-trailers (including Euclids)
Class 6: Heavy duty trailer-40 tons and over

SUPERSEDEAS DECISION

STATE: Vermont COUNTIES: Chittenden, Franklin, Grand Isle, Addison, & Orleans
DECISION NO. VT78-2067
Supersedes Decision No. VT76-2170 (above counties only) dated December 10, 1976 in 41 FR 54146. DATE: Date of publication.
DESCRIPTION OF WORK: Highway Construction

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
Chittenden, Franklin, Grand Isle, and Orleans Counties:					
Carpenters	$6.65	.35	.30		
Cement masons	7.63				
Laborers:					
Laborers	3.85	.15	a		
Drillers: Chittenden County	4.40				
Remainder of Counties	4.69				
Truck Drivers:					
2 axle	5.35				
3 axle	5.50				
Power equipment operators:					
Augers	5.50				
Backhoe	8.20	.35	.50	b	.02
Bulldozer	7.75	.35	.50	b	.02
Compactor:					
Orleans	7.20	.35	.50	b	.02
Franklin, Grand Isle, & Chittenden	7.75	.35	.50	b	.02
Crane	8.20	.35	.50	b	.02
Gradall	8.20	.35	.50	b	.02
Mechanic	7.75	.35	.50	b	.02
Paver	7.75	.35	.50	b	.02
Roller	7.75	.35	.50	b	.02
Loader	7.75	.35	.50	b	.02
Spreader (Orleans County only)	7.75	.35	.50	b	.02
Grader:					
Chittenden Co.	4.30				
Remainder of Counties	7.95	.35	.50	b	.02

Footnotes:
a. 2 paid holidays: Memorial Day, Independence Day, provided the employee has been employed for at least 7 days or more prior to the holiday and has worked 2 full days in the calendar week in which the holiday falls.

b. 9 paid holidays: New Year's Day; Memorial Day, Independence Day, Labor Day, Thanksgiving Day, Christmas Day, Washington's Birthday, Columbus Day, and Veteran's Day.

DECISION NO. VT78-2067

	Basic Hourly Rates	Fringe Benefits Payments			
		H & W	Pensions	Vacation	Education and/or Appr. Tr.
Addison County:					
Carpenters	$6.65	.35	.30		
Cement masons	6.65	.35	.30		
Ironworkers:					
Reinforcing	6.46	.25			.05
Structural	7.40	.45	.60		
Laborers	3.85	.15		a	
Driller	4.48				
Asphalt [illegible]	4.35				
Air tool operator	4.10				
Truck drivers:					
2 axle	5.35				
3 axle	5.50				
Power equipment operators:					
Roller	7.75	.35	.50	b	.05
Grader	7.95	.35	.50	b	.05
Mechanic	7.75	.35	.50	b	.05
Paver	7.75	.35	.50	b	.05
Backhoe	8.20	.35	.50	b	.05
Bulldozer	7.75	.35	.50	b	.05
Crance	8.20	.35	.50	b	.05
Front end loader	7.75	.35	.50	b	.05
Spreader	7.75	.35	.50	b	.05
Compactor roller	7.20	.35	.50	b	.05

Footnotes:

a. 2 paid holidays-- Memorial Day and Independ. Day, provided the worker worked 2 full days in the calendar week in which the holiday falls.

b. 9 paid holidays-- Ney Years Day, Memorial Day, Independence Day, Labor Day, Thanksgiving Day, Christmas, Washington's Birthday, Columbus Day, Veterans Day.

[FR Doc 78-20679 Filed 7-27-78; 8:45 am]

federal register

FRIDAY, JULY 28, 1978
PART IV

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

National Institutes of Health

■

RECOMBINANT DNA RESEARCH

Proposed Revised Guidelines

[4110-08]

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

National Institutes of Health

RECOMBINANT DNA RESEARCH

Proposed Revised Guidelines

STATEMENT BY JOSEPH A. CALIFANO, JR., SECRETARY OF HEALTH, EDUCATION, AND WELFARE

The Director of the National Institutes of Health is publishing proposed revisions to the NIH guidelines on research involving recombinant DNA molecules.

The original guidelines are being updated in light of NIH's experience operating under them and in light of our increasing knowledge about the potential risks and benefits of this research technique. As experience accumulates, we should review and evaluate the evidence to assure that the restrictions imposed are appropriate to potential risks—strengthening restrictions where needed, relaxing regulation where justified.

In publishing these proposals for public comment, I recognize the extraordinarily difficult challenge that developing sensitive but effective regulations in this field poses for NIH, for the research community, and for the concerned public.

Necessarily, this task poses difficult questions that we will never be able to answer with complete certainty. I hope that those concerned will analyze the proposed revisions with care and give us their views on the strength of the evidence that supports the proposed revisions, the specific scientific and research containment procedures that the proposed revisions require, and the procedures and the standards they establish for future changes in the guidelines and for the exercise of discretion under them.

We particularly seek comment on the sections in the proposed revisions that establish the mechanisms for administering and revising the guidelines. For example, do the proposed revisions strike the proper balance in establishing:

The procedures for permitting otherwise prohibited experiments and for exempting classes of research from the guidelines;

The standards for the exercise of administrative discretion under the guidelines;

The composition of the Department's Recombinant DNA Advisory Committee and of the institutional biohazard committees.

To review the comments on the proposed revisions, I am establishing a departmental review committee, consisting of Mr. Peter Libassi, the Department's General Counsel (Chairperson); Dr. Donald Fredrickson, the Director of NIH (Vice-Chairperson); Dr. Julius Richmond, Assistant Secretary for Health; and Dr. Henry Aaron, Assistant Secretary for Planning and Evaluation.

I have asked this committee to hold a public hearing to insure full and complete opportunity for comment. In order to hold this hearing and to issue the revised guidelines on a reasonably prompt schedule, no extension of the 60-day period for public comment will be possible.

In preparing these revisions, the National Institutes of Health have already held 19 hours of public hearings and have received continual advice from the scientific community and from the public. I want this open process to continue. I urge those concerned to help us find the proper balance by providing us with their comments on NIH's proposed revisions.

Dated: July 19, 1978.

JOSEPH A. CALIFANO, JR.,
Secretary.

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

NATIONAL INSTITUTES OF HEALTH

RECOMBINANT DNA RESEARCH

PROPOSED REVISED GUIDELINES—NIH

This will introduce three related documents that the National Institutes of Health (NIH) is publishing for public comment: (1) a *Decision of the Director, NIH,* to publish revised NIH guidelines for research involving recombinant DNA molecules, (2) the *Proposed Revised Guidelines—NIH,* and (3) an *Environmental Impact Assessment* of the proposed action. The Secretary of Health, Education, and Welfare has approved the release of these documents for public comment.

As stated in the Secretary's preface, we are particularly concerned that public comment be invited on the scientific and procedural aspects of the proposed guidelines. A public hearing on these proposed revisions will be held in late September at the Hubert H. Humphrey Building, Washington, D.C. All comments received on or before September 25, 1978, will be considered, and no extension of the comment period will be granted. Within 45 days after the comment period, final guidelines will be promulgated with a notice in the FEDERAL REGISTER.

The events leading to the proposed revisions are described in the "Introduction and Overview" to the *Decision* and in the "Foreword" to the *Assessment.* This preface will orient the reader to other NIH documents that are directly related to the present publications. One is the current *Recombinant DNA Research Guidelines,* effective since June 23, 1976 (published in the FEDERAL REGISTER, July 7, 1976). Another is the *Environmental Impact Statement* (EIS) on those guidelines, published in 1977. The EIS, which contains a copy of the guidelines, is available from the Government Printing Office (stock No. 017-040-00413-3) and in GPO depository libraries throughout the country. A third related document is *Proposed Revised Guidelines on Recombinant DNA Research,* which the NIH Recombinant DNA Advisory Committee (RAC) recommended to the NIH Director on September 1, 1977. The RAC proposal was published in the FEDERAL REGISTER, September 27, 1977, for public comment.

The RAC-proposed revisions were discussed at a public meeting of the Advisory Committee to the Director (DAC), held at NIH on December 15-16, 1977. The DAC and special consultants heard witnesses from environmental groups, the scientific community, industry, etc. All correspondence from the public in response in the FEDERAL REGISTER publication was available to those present and will be published, with the transcript of the meeting and other documents, as part of a continuing public record of NIH activities concerning recombinant DNA. The Director, NIH, acting in light of the 2-day discussion, all commentaries, and the DAC's recommendations, has arrived at the present proposal—the *Proposed Revised Guidelines* (NIH)—and offers it for public review.

The current (1976) guidelines contain an appendix entitled "Supplementary Information on Physical Containment." This has been omitted from the present proposed guidelines but, in revised and expanded form, will be available on request as "Laboratory Safety Monograph—A Supplement to the NIH Guidelines for Recombinant DNA Research." For a copy, write to the Office of Recombinant DNA Activities, Building 31, Room 4A52, National Institutes of Health, Bethesda, Md. 20014.

The *Environmental Impact Assessment* is based on an intensive analysis of the current guidelines, the RAC-proposed alternative, and the present NIH alternative. The conclusion of this analysis is that there would be no adverse impact of the NIH-proposed changes upon the environment.

The guidelines as presently proposed are designed to discharge the continuing obligation of NIH to insure that recombinant DNA research goes forward under standards of safety reflecting the latest scientific knowledge, so that the public and the environment are protected from any hazards while deriving the full benefits of the recombinant DNA technique.

Written comments and inquiries concerning the *Proposed Revised Guidelines* should be addressed to the Director, National Institutes of Health, Bethesda, Md. 20014. All comments received will be available for public inspection at the Director's office on weekdays (Federal holidays excepted) between the hours of 8:30 a.m. and 5 p.m.

Dated: July 19, 1978.

DONALD S. FREDRICKSON,
Director,
National Institutes of Health.

DECISION OF THE DIRECTOR, NATIONAL INSTITUTES OF HEALTH, TO ISSUE REVISED GUIDELINES FOR RECOMBINANT DNA RESEARCH

JULY 19, 1978.

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INTRODUCTION AND OVERVIEW

Today, with the concurrence of the Secretary of Health, Education, and Welfare, and the Assistant Secretary for Health, I am proposing revisions to the NIH Guidelines for Recombinant DNA Research.(*1*) These Guidelines were first issued on June 23, 1976. The proposed revisions result from a continuing process of scientific and public exchange similar to that of the 1976 edition. This overview sketches the background for proposed revisions and summarizes the proposed changes. It references accompanying documents and other pertinent sources of information.

The probable risks and benefits of recombinant DNA research—the larger subject of which the NIH Guidelines are a part—have been discussed in numerous forums since first addressed in 1973.(*2*) Congress has held multiple hearings on related issues, including proposals to convert the Guidelines to Federal regulations(*3*) and redefine recombinant DNA research to narrow the range of experiments subject to regulations. Early in 1977 the NIH Recombinant DNA Advisory Committee (RAC), the scientific and technical committee responsible for proposing revisions to the Guidelines, began its task of identifying changes needed in the Guidelines and forwarded suggestions to me for consideration. In order that public comments could be heard on the RAC-proposed revisions, published in September 1977,(*4*) a meeting of the Advisory Committee to the Director, NIH (DAC), the committee responsible for public oversight, was held in December. The extensive record of this hearing bears witness to almost unanimous agreement that the original Guidelines badly need updating, and suggests numerous directions in which revisions might move.(*5*)

Much of the discussion at the December 1977 meeting of the DAC affirmed the need for continuous reevaluation of the scientific premises underlying the original Guidelines. Since Asilomar,(*2*) growing evidence has suggested that other experts ought to review the concerns of the molecular biologists who first raised questions about the safety of recombinant DNA research. Scrutiny from experts in infectious diseases, epidemiology, virology, botany, ecology, laboratory safety, and other disciplines has been needed. NIH sponsored a workshop for this purpose at Falmouth, Mass., 6 months before the DAC meeting. Here, old and new information about *E. coli* K-12—the host most used in recombinant DNA experiments—was interpreted carefully. From this came a consensus that the chances of this host being convertible to an epidemic pathogen are negligible.

Those attending the December DAC meeting also heard complaints that containment levels were set too stringently for recombinant DNA work on viruses and plants. This applied both to the original Guidelines and to the revisions proposed in September 1977. A decision was made to address the issues through workshops without delay.

One of these workshops, held at Ascot, England, dealt specifically with viruses.(*7*) Here, experts from several countries, most of whom had no stake in recombinant DNA experiments, reached an unequivocal opinion that the risks of cloning viral DNA in a bacterium like *E. coli* K-12 are not greater, and are usually much less, than the risks of handling the parent virus alone. They also stressed that defective viruses pose little risk of infection when used as vectors for cloning DNA in eukaryotic cells, since the cells cannot survive outside permissive laboratory conditions and the virus cannot escape in a viable form. A second working group, meeting in Bethesda,(*7*) then reviewed the conclusions. It agreed that the original Guidelines imposed stricter containment on use of viral DNA or of viruses as vectors than could be justified by any available fact, and recommended changes.

A group of agricultural scientists met in Washington, D.C., in March 1978(*8*) to consider the containment conditions for incorporation of DNA from plant pathogens into *E. coli* K-12 and for use of viral and other vectors in plants. An important concept discussed at this workshop is the lack of evidence that *E. coli* K-12, or any other strain of this bacterium, is capable of acquiring an ecological niche in plants and thus infecting them.

On April 27-28, 1978, the RAC considered the recommendations concerning viruses and plants and agreed with most of them. With a few changes, they are a part of the proposed revision.

At the December 1977 hearings before the DAC, other aspects of the Guidelines evoked requests for revisions.(*5*) There was overwhelming sentiment for exempting from the Guidelines experiments involving recombination of DNA within the same strains or from pairs of organisms that transfer genes in nature. Discretion to exempt such experiments is not provided in the original Guidelines; nor does one find there the flexibility to permit other experiments for purposes of risk assessments needed to determine the merits of particular standards, or how these should be revised. Other criticisms of the Guidelines stressed the delays and confusion created by excessive centralization of administrative control, and it is evident that implementing procedures must be changed.

Certain background elements merit comment. Shortly after the NIH Guidelines were published, the British guidelines appeared.(*9*) Subsequently other national guidelines have been issued, most recently those of the Soviet Union. Many international aspects of DNA research and its regulation have been reviewed elsewhere.(*9*) The December 1977 DAC meeting directed attention to instances where the NIH Guidelines either exclude experiments that have been conducted abroad or make them far more diffi-

cult to do.(*10*) Moreover, factual bases for the greater stringency of the U.S. (NIH) Guidelines cannot be shown.

Five years have passed since concerns were first raised about the hypothetical hazards of laboratory experiments with recombinant DNA. The thousands of individual applications of such techniques have produced much useful knowledge, but no evidence has come to light of a product created by these techniques that has been harmful to man or the environment. Foreign genes inserted into prokaryotic host-vector systems have been faithfully replicated and produced in quantities valuable to science. On the other hand, prokaryotes generally have not been able to translate eukaryotic genes into biologically active proteins. No new facts or unconsidered older ones have emerged to support the fears of harmful effects, and one prominent early proponent of guidelines has repudiated his support for them.(*11*) At the least, there is growing sentiment that the burden of proof is shifting toward those who would restrict recombinant DNA research.(*12*)

Although clearly the time has come to revise the original NIH Guidelines for Recombinant DNA Research, it is not the time to conclude that they are being altered in preparation for their early abandonment. Understanding of gene regulation and expression is increasing inexorably and at an awesome pace. We may predict that ways will be found to achieve and control the translation of foreign genes by a variety of hosts.(*13*) As the barriers to translation are dropped, some of the larger promise of recombinant technology will be realized. In some proportion to the harvest of positive results, a capability must be maintained for observing any capacity of these experiments to yield harmful products, and for communicating this to all who have an interest in similar experiments.

In preparation for this next phase of recombinant DNA research, several shifts in NIH guidance are necessary. Experiments posing no threat to safety must be exempted from the Guidelines; and provisions must be made to remove others as soon as their harmlessness becomes evident. Any universal rules imposed on this kind of activity derive validity from continual modification dictated by results of the experimentation they govern.

Primary responsibility for comliance with the rules must be located where the work is done. There it must be shared fully by principal investigators, those who work in their laboratories, institutional biosafety committees, and the institutional leaders. The NIH Office of Recombinant DNA Activities (ORDA) should be relieved of its burden of obligatory prior approval of certain experiments, so that it can better carry out, along with the RAC, two central functions. These are the continuing synthesis and interpretation of the Guidelines, and the maintenance of full communication among all who must use them.

To recapitulate, these new proposed Guidelines arose from a proposal made to me by the RAC in September 1977. Numerous amendments have been made on the basis of public comments received at the December 1977 hearing, in extensive correspondence before and after that, and recommendations of special expert workshops whose reports were then assessed by the RAC in April 1978. The proposal and the amendments have been the products of long and intense participation by numerous persons representing many points of view. I now summarize the more important proposed changes. The basis for decision on each element of revision is provided in detail in subsequent sections of this document.

SCOPE AND APPLICABILITY OF THE GUIDELINES

Recombinant DNA containing synthetic sequences is now explicitly part of the definition of what is included under the Guidelines. The standards of the Guidelines now apply to all recombinant DNA experiments conducted in an institution that receives any support from NIH for recombinant DNA research. This includes a registration requirement.

The original Guidelines contain a number of prohibited experiments. There was little sentiment for the removal of all the original prohibitions—although it has been noted that the U.S. (NIH) Guidelines are the only national guidelines to stipulate "prohibited" activities. The original prohibitions, with one modification and a necessary "flexibility" clause,(*14*) are therefore retained in the proposed revision. They immediately precede a new section called "Exemptions"—a juxtaposition chosen to emphasize that the prohibitions still override.

The first exemption from the guidelines covers the handling of DNA outside a host organism or virus. Such "naked DNA" has been handled in laboratories for years and is rapidly inactivated in nature.

The exempted experiments of the second class consist essentially in rearranging, or deleting from, molecules of nonchromosomal or viral DNA. No foreign DNA is involved. An example would be the introduction of a DNA molecule formed from pieces of SV40 virus into eukaryotic cells in tissue culture. Since there is little if any basis for presuming such "rearrangement" or "deletion" experiments to be hazardous, they are now excluded.

A third class of exemptions are experiments called "self-cloning," in which DNA found naturally in a host may be reinserted into that host. These are reproductions in the laboratory of events that occur in nature.

Similarly, provision is made in the proposed guidelines for exemption of a fourth class of experiments that involve donor-host pairs that normally exchange DNA. Such genetic exchange is known to occur widely between various species of bacteria and is generally mediated by certain plasmids or viruses. Experimental recombinations of this type are only an imitation of what nature is able to accomplish handily in the absence of Federal regulation. A list of donor-host pairs to be exempted is begun in this revision and will be expanded periodically as knowledge grows. The initial choice from several possible lists submitted to me by the RAC is a conservative one, restricted to pairs of organisms for which there is documented evidence of natural exchange.

Finally, a fifth exemption is provided for removal of other recombinations when they are shown to be safe. The last two exemptions create some of the discretionary power for modifying the guidelines that was so lacking in the original. Provision will be made for public input to such decisions, either by announcement of proposed exemptions prior to consideration by the RAC or before a decision by the Director becomes effective.

CONTAINMENT

I have made one decision that will not be regarded with equal pleasure by all engaged in recombinant DNA research. P1 containment previously permitted mouth pipetting. In accord with a previous recommendation by the European Molecular Biology Organization (EMBO), its virus Working Group strongly recommended prohibiting this practice; and so did NIH safety advisors. The RAC at its meeting on April 27-28, 1978, recommended that mouth pipetting be prohibited only for those P1 recombinant DNA experiments involving viral DNA. Rather than create two separate classes of P1, and in recognition of the present availability of excellent mechanical devices for pipetting, I am proposing that mouth pipetting no longer be permitted in P1 containment. Since it is already prohibited in P2-P4 containment, this bans the use of mouth pipetting for any experiment covered by the Guidelines.

CONTAINMENT GUIDELINES FOR COVERED EXPERIMENTS

The recommendations of the RAC, arising from the Ascot-Bethesda work-

shops, represent the first realistic appraisal of any hazards that might lie in the use of viral vectors or the cloning of viral DNA. Recombinant techniques offer access to ares of viral biology that are vitally important. Such studies should not be impeded unnecessarily. I have accepted the April 1978 recommendations of the RAC in this area with minor amendments. The revised guidelines emphasize the current dictum that any hazards of working with viruses in recombinant DNA experiments are maximal at the first stage, when the virus itself with its full genomic complement is handled.

The RAC unanimously approved modest changes in containment for plant experiments. I have also approved them provisionally, contingent upon concurrence by the Department of Agriculture.

A new sentence has been added to the guidelines giving much needed flexibility in the setting of containment levels.(*15*)

ROLES AND RESPONSIBILITIES

Two years' experience with the guidelines has offered valuable tutelage in the limits of external (Federal) control of laboratory experimentation. Scientists and their co-workers have long experimented with pathogenic organisms, poisonous plants and animals, and hazardous chemicals. The laboratory is not among the more notorious occupational settings for accidents or illness, and damage to community or environment by basic laboratory research is almost unknown. Control over the use of radioisotopes in the laboratory, long a Federal preserve, is not comparable to use of recombinant DNA techniques; for the risks of using radioisotopes are calculable and mistakes are easily measured. Thus realistic and durable standards can be set. Without a base for the setting of such standards, conventional regulation is difficult at best, and at worst can be preposterous.

In the case of recombinant DNA technology, we are in the midst of a search for any risks, and thus for applicable standards. The scientists who raised the possibility of risks also realized that the only effective safeguards lay in a maximum enhancement of the collective nature of the scientific process. The usual communications networks of science had to be augmented and the evaluation of results and the reaching of consensus accelerated. These actions, is was reasoned, would help establish a set of initial rules, and there was the added assumption that they could and should be kept up to date. All using the new techniques would sign a "memorandum of understanding" to the effect that, until things became clearer, the basic communality of scientific inquiry would be especially emphasized in any work with recombinant DNA techniques.

The power of the Government to require such discipline of its grantees was an attractive reason for the scientists to request Federal intervention. And the Federal capacity to achieve the essential communication and consensus-building has been one of the most positive results of this experiment in adminstration. But the price of Fedeal intervention includes a heavy tax of formalism. In the instance of these guidelines, diverse pressures have made difficult the appropriate balancing of substance and procedure. I have already alluded to one of the undesirable results—a chilling inflexibility of the original guidelines—and its proposed correction by revision.

Prior NIH clearance is mandatory for new NIH grants and contracts involving recombinant DNA techniques and for all projects in P4 facilities. In the proposed revised guidelines, prior NIH clearance is no longer required for changes at the P1–P3 levels. These changes must be approved by the institutional biosafety committee (IBC), and NIH will then review the IBC actions. This proposal reverses an October 1977 issuance stating that changes in ongoing projects require prior NIH clearance. The requirement resulted in numerous delays in projects which could not be justified on grounds of safety.

The proposed guidelines would strengthen institutional responsibilities and authorities in determining compliance. A full partnership with all investigators and their institutions is intended. The role of the IBC is particularly enhanced through delegation of some discretionary powers that were previously reserved for NIH and the RAC. To better meet these obligations, an institution using P3 or P4 containmen is required under the proposed guidelines to have a qualified biological safety officer.

Experience gained in the past 5 years in explaining recombinant DNA technology has shown how valuable can be a community's activities. At least one member of the IBC is to be a "public member"—i.e., one who has no financial connection with the institution. Further, to ensure opportunity for public particiation at the national level, procedural are set forth, as explained in Part IV of the decision, that provide public notice and solicit comment on the major actions of NIAH.

Another stipulation of the revised guidelines is that failure of compliance can lead to suspension of NIH support for recombinant DNA research.(*16*)

Provision is now made for the private sector to register voluntarily its recombinant DNA activities with NIH. Also, other consulting services, including certification of host-vector systems, will be provided. The service will be accompanied by protection of proprietary data as mandated by law.

NIH issued a draft environmental impact statement on the guidelines in September 1976. This was revised after public comment and issued in final form in October 1977. It concluded that the activities covered by the guidelines had no predictable impact on the environment, since all the risks discussed were hypothetical. The EIS was examined by a Federal district court in 1978.(*17*)

In parallel with the process of revising the guidelines, NIH has conducted an environmental impact assessment, including an analysis of how current experiments supported by NIH will be affected by this revision. Again, the activities covered by the revised guidelines deal only with hypothetical risks, and thus the assessment reveals no predictable impact on the environment. Its content is published herewith in a companion document.

ORGANIZATION OF THE REMAINDER OF THIS DOCUMENT AND ABBREVIATIONS USED

The Recombinant DNA Molecule Program Advisory Committee is somnetimes referred to below as the Recombinant DNA Advisory Committee or Recombinant Advisory Committee or RAC.

The meeting of the Advisory Committee to the Director, NIH, which took place in December 1977 is sometimes referred to below as the meeting of the Director's Advisory Committee or of the DAC or the December 1977 public hearing.

The "NIH Guidelines for Research Involving Recombinant DNA Molecules" as issued on June 23, 1976, and publishes in the FEDERAL REGISTER on July 7, 1976, are sometimes referred to below as the original guidelines or the 1976 guidelines or the current guidelines.

The proposed revised guidelines prepared by the RAC and published in the FEDERAL REGISTER on September 27, 1977, are referred to below as the PRG-RAC.

The proposed revised guidelines which are being proposed now by NIH are referred to below as the PRG-NIH.

The remainder of this document is divided into four parts corresponding to the four parts of the guidelines; i.e., I. Scope of the Guidelines; II. Containment; III. Containment Guidelines for Covered Experiments; and IV. Roles and Responsibilities.

Within each of these four parts there are two subsections; i.e., Review of RAC-Proposed Guidelines and Review of Comments and NIH-Proposed Guidelines. The first subsection

describes how the PRG-RAC differs from the 1976 guidelines; the second describes (1) the public comments received both before and after the December 1977 DAC meeting, concerning the PRG-RAC, and (2) the changes which have been made in response to these comments leading to the PRG-NIH.

FOOTNOTES TO INTRODUCTION AND OVERVIEW

(1) In addition to the proposed revised guidelines and this "Decision Document," there is also being released an Environmental Impact Assesemment, including numerous appendices.

(2) The capability to perform DNA recombinations, and the potential hazards, had become apparent to scientists at the Gordon Research Conference on Nucleic Acids in July 1973. At their behest the National Academy of Sciences created a committee that organized an international conference held in February 1975 at Asilomar Conference Center, Pacific Grover, Calif. Approximately 150 scientists, of whom a third were from foreign countries, were present. The committee also called on the National Institutes of Health to establish an advisory committee to draft guidelines for the conduct of this research. Temporary guidelines were issued at Asilomar pending issuance of NIH guidelines.

In response, the NIH Recombinant Advisory Committee (formally "NIH Recombinant DNA Molecule Program Advisory Committee") was established in October 1974 to advise the Secretary of HEW, the Assistant Secretary for Health, and the Director of NIH to accomplish these tasks. The several meetings at which the Recombinant Advisory Committee developed its proposed guidelines in 1975 were announced in the FEDERAL REGISTER and were open to the public. The committee, after preparing several draft versions of guidelines, reached agreement on a recommended revised version, which was referred to the NIH Director for review in December 1975.

A special meeting of the public advisory Committee to the Director, NIH, was convened in February 1976 to review these proposed guidelines. In addition to current members of the committee, a number of former committee members as well as other scientific and public representatives had been invited to participate. There was ample opportunity for comment and an airing of the issues, both by the committee members and the public witnesses. All major points of view were broadly represented.

The proposed guidelines were reviewed by the Director, NIH, in the light of comments and suggestions made at the public hearing as well as extensive written correspondence received after the meeting. When the final guidelines were released in June 1976, an accompanying decision paper described in great detail all relevant public comments and the reason for accepting or rejecting specific recommendations in preparing the final guidelines. The NIH guidelines and the Decision of the Director, NIH, were published in the FEDERAL REGISTER on July 7, 1976. In addition, copies of the guidelines were widely distributed to foreign embassies, medical and scientific journals, NIH grantees and contractors, and professional research societies.

(3) The following committees have held hearings and/or markup sessions on Recombinant DNA legislation:

House—The Subcommittee on Health and the Environment and its parent, the Committee on Interstate and Foreign Commerce; the Subcommittee on Science, Research, and Technology and its parent, the Committee on Science and Technology.

Senate—The Subcommittee on Health and Scientific Research and its parent, the Committee on Human Resources; the Subcommittee on Science, Technology, and Space. Its parent, the Committee on Commerce, Science, and Transportation, has not held any hearings or markup sessions on this topic.

	Bill	Chief sponsor	Date
House:			
H. Res. 131		Richard Ottinger, Democrat of New York.	Jan. 19, 1977.
H.R. 3191	Identical to S. 621	do	Feb. 7, 1977.
H.R. 3591	do	do	Feb. 16, 1977.
H.R. 3592	do	do	Do.
H.R. 4232		Stephen Solarz, Democrat of New York.	Mar. 1, 1977.
H.R. 4759		Paul Rogers, Democrat of FLorida	Mar. 9, 1977.
H.R. 4849	Identical to H.R. 4759	do	Mar. 10, 1977.
H.R. 5020	Identical to S. 621	Ottinger	Mar. 14, 1977.
H.R. 6158	Administration bill	Rogers	Apr. 6, 1977.
H.R. 7418		do	May 24, 1977
H.R. 7897		do	June 20, 1977.
H.R. 11192		Rogers and Harley Staggers, Democrats of West Virginia.	Feb. 28, 1978.
Senate:			
S. 621		Dale Bumpers, Democrat of Arkansas.	Feb. 4, 1977.
S. 945		Howard Metzenbaum, Democrat of Ohio.	Mar. 8, 1977.
S. 1217	Administration bill	Edward Kennedy, Democrat of Massachusetts.	Apr. 1, 1977
S. 1217	As reported; accompanied by Report 95-359.	do	July 22, 1977.
S. 1217	Amendment 754 in the nature of a substitute.	Gaylord Nelson, Democrat of Wisconsin.	Aug. 2, 1977.
S. 1217	Amendment 1713 in the nature of a substitute.	Kennedy	Mar. 1, 1978

The following bills on recombinant DNA technology have been formally introduced:

(4) The Recombinant Advisory Committee considered its proposed revisions at meetings throughout 1977. The version proposed to the Director, NIH, in September 1977, appeared in the FEDERAL REGISTER on September 27, 1977.

(5) This meeting of the Director's Advisory Committee took place in Bethesda on December 15-16, 1977. A summary of the meeting appeared in the recombinant DNA technical bulletin, and the complete record will shortly be published by NIH in vol. 3 of the series recombinant DNA research.

(6) The NIH-sponsored meeting at Falmouth, Mass., on June 20-22, 1977, was chaired by Dr. Sherwood Gorbach. A complete record of this meeting appears in the "Journal of Infectious Diseases" (May 1978).

(7) The "U.S.-EMBO Workshop to Assess Risks for Recombinant DNA Experiments Involving the Genomes of Animal, Plant, and Insect Viruses" was held on January 26-28, 1978, in Ascot, England. It was attended by experts on viruses from the United States, Britain, and other European countries, a majority of whom were not engaged in recombinant DNA research. The primary purpose of the meeting was to conduct a scientific and technical analysis of possible risks associated with cloning eukaryotic viral DNA segments in *E. coli* K-12 host-vector systems and with the use of eukaryotic viruses as cloning vectors in animal, plant, and insect systems. The report of the workshop was published in the FEDERAL REGISTER on March 31, 1978, and appears as appendix E to the accompanying environmental impact assessment. The results of the Ascot meeting were then reviewed by another group of U.S. virologists who converted them into recommendations for revision of the guidelines. This working group was chaired by Dr. Harold Ginsberg and met on April 6-7, 1978. Its report appears as appendix F to the accompanying environmental impact assessment. The report was considered by the Recombinant Advisory Committee at its April 27-28, 1978, meeting.

(8) The "Workshop on Risk Assessment of Agricultural Pathogens" was held on March 20-21, 1978, in Washington, D.C., under the auspices of the National Science Foundation, the Department of Agriculture, and the National Institutes of Health. A copy of the report of this workshop appears as appendix G to the accompanying environmental impact assessment.

(9) The United Kingdom guidelines, also known as the "Williams report," were issued in August 1976. A fairly comprehensive review of the international aspects of recombinant DNA research, including issuance of national guidelines, is contained in the "Report of the Federal Interagency Committee on Recombinant DNA Research: International Activities," November 1977. This is available from the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Md. 20014.

(10) Under the NIH guidelines, experiments using prokaryotic hosts other than *E. coli* K-12 are severely limited whereas such experiments are proceeding in Europe, especially with *Bacillus subtilis*. Certain other categories of experiments require, according to the NIH guidelines, either P4+EK2 or P3+EK3 containment. Since no EK3 system

has as yet been certified and since the first P4 facility has only recently been certified, these experiments were effectively forbidden. The same experiments require significantly lower containment under some European guidelines.

(*11*) Prof. James Watson, in testimony at the December 1977 DAC meeting and in print, has sought repentance for his earlier activities in support of special precautions for recombinant DNA research.

(*12*) The report, "Science Policy Implications of DNA Recombinant Molecule Research," March 1978, of the Subcommittee on Science, Research, and Technology of the Committee on Science and Technology, U.S. House of Representatives, says, "The burden of proof of safety factors should not be borne exclusively by proponents of recombinant DNA research; opponents must assume a corresponding burden."

(*13*) Significant differences exist between prokaryotes and eukaryotes in the ways proteins are synthesized under genic direction, and these account for limitations on the apparent success of many recombinant DNA experiments to date. A major thrust of current recombinant DNA research is in the direction of overcoming these differences. There is every reason to believe that this research will succeed. At my invitation, Dr. Malcolm Martin of NIH has drawn up this brief analysis of the state-of-the-art:

The potential use of recombinant DNA techniques to produce biologically useful reagents is predicated on: (a) the faithful replication of a segment of foreign DNA in a new host cell; (b) the synthesis of messenger RNA (mRNA) complementary to the inserted DNA; and (c) the efficient translation of the mRNA into a polypeptide. In nearly all cases that have been examined to date, DNA, from both eukaryotic and prokaryotic sources, has been amplified in prokaryotic host-vector systems. The fidelity of this entire process (a, b, and c) has been verified in several instances in which prokaryotic DNA segments have been cloned in *E. coli* and resulted in the synthesis of new polypeptides. Thus, in such cases, the informational content contained in the inserted prokaryotic DNA is expressed as evidenced by the synthesis of mRNA and novel proteins.

With few exceptions (some yeast inserts) the expression of eukaryotic DNA in the form of biologically active or biochemically detectable polypeptides in prokaryotes has not been demonstrated using chromosomal DNA inserts and unmodified vectors. In nearly all cases where the system has been rigorously examined, it has been shown that eukaryotic DNA has replicated in *E. coli;* in some instances, RNA complementary to the inserted eukaryotic DNA has been identified.

Messenger RNA synthesis and function in E. coli. The synthesis of messenger RNA (mRNA) in a prokaryote, such as *E. coli*, proceeds in a linear fashion along the DNA template of individual gene segments or groups of related genes. In nearly all cases examined, the mRNA molecules are the faithful colinear transcripts of prokaryotic genetic information and can be used in an unmodified form to direct the synthesis of prokaryotic polypeptides. The informational content of mRNA corresponds directly to the nucleotide sequence of DNA in such systems (i.e., *all* nucleotides present in a prokaryotic gene are transcribed into messenger RNA which, in turn, programs the synthesis of a corresponding protein). Control of this phase of gene expression appears to be solely at the level of RNA synthesis.

In prokaryotes (and eukaryotes), nucleotide sequences preceding the sequences corresponding to the actual genes play a major role in determining (a) whether a given DNA sequence will be transcribed into RNA and (b) whether the RNA so synthesized will efficiently bind to ribosomes, a prerequisite for protein synthesis. For example, certain DNA sequences interact with regions of RNA polymerase and thereby participate in the initiation of RNA synthesis; they are not represented in the final RNA product. DNA sequences specifying binding to ribosomes are physically located between those for initiation of RNA synthesis and sequences encoding the amino acids of a particular protein (the gene) and are also contained in the functional mRNA molecules.

Messenger RNA synthesis and metabolism in eukaryotes. Our understanding of gene regulation and expression in eukaryotic cells has increased markedly during the past 10 months. A common feature of all systems that have been carefully evaluated is that the initial, faithful RNA copy of the DNA is extensively modified to produce a functional form of mRNA. The final mRNA contains only a fraction of the sequences present in the original RNA product. That is to say, portions of large RNA molecules are removed by mechanisms that are, at present, poorly understood and the remaining segments of the primary RNA transcript are then rejoined to one another. In nearly all cases an RNA segment containing a ribosomal binding site is joined to a segment coding for a polypeptide; in addition, larger gene segments are often joined together. This process was first observed in animal virus systems (1, 2) where it was shown that viral mRNA, containing the information for a product which had been previously mapped to a specific locus on the viral genome, was complementary to regions of the viral DNA which were separated by more than a thousand nucleotides.

Support for the concept of complex modification leading to functional mRNA in eukaryotic cells has recently come from recombinant DNA experiments in which chromosomal DNA has been cloned in *E. coli*. When individual cloned eukaryotic genes are carefully analyzed, intervening DNA sequences which interrupt the actual sequence of the gene in chromosomal DNA have been identified. To date, such intragenic DNA has been detected in ovalbumin (3, 4), β globin (5, 6), immunoglobulin (7), and even tRNA genes (8). In one instance it has been clearly shown that the intervening DNA sequences, present in the primary RNA transcript of β globin DNA, are absent in β globin mRNA (5). These mechanisms presumably function in some regulatory fashion to modulate eukaryotic gene activity.

Implications for Recombinant DNA Research

A. The discovery of the existence of complex processes involved in the maturation of mRNA eukaryotic cells and the demonstration of intragenic DNA in several eukaryotic genes suggests that: (1) cloning of chromosomal DNA in *E. coli* DNA (shotgun or purified) will pose little, if any, risk since the maturation mechanisms have never been observed in prokaryotes; and (2) investigators who wish to develop prokaryotic cloning systems for the purpose of synthesizing useful biological products will utilize cDNA copies of functional mRNAs or synthetic DNA with a nucleotide sequence derived from a known amino acid sequence as DNA inserts.

B. Vectors are currently being "engineered" to ensure efficient transcription and translation of DNA inserts. Using slightly different approaches, groups in San Franscisco and at Harvard (9-11) are preparing DNA segments which: (1) contain the sequences necessary for interaction with *E. coli* RNA polymerase linked closely to (2) sequences which encode a bacterial ribosome binding site. Such DNA segments can then be added to a prokaryotic cloning vector next to the site into which a foreign DNA will be inserted. This arrangement will facilitate the transcription of the inserted DNA and enable the mRNA so synthesized to bind to bacterial ribosomes. This embellishment has already been used to maximize the expression of a bacteriophage gene and human somatostatin DNA in a plasmid vector system (10, 11).

References for Footnote 13

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(*14*) Prohibition (1) in the original guidelines forbids experiments with "oncogenic viruses classified by NCI as moderate risk." The absence of evidence that use of these viruses will lead to formation of agents harmful to man and the potential for obtaining useful new knowledge, relevant to carcinogenesis in particular, and genetics in general, supports the removal of the prohi-

bition. The removal of this prohibition was proposed in the report of the Working Group on Viruses which met on Apr. 6-8, 1978, and endorsed by the RAC at its Apr. 27-28, 1978, meeting. The reasoning behind this is that recombinant DNA experiments with pieces of these viruses cloned in *E. coli* K-12 pose no more risk, and actually appear to pose clearly less risk, than work with the whole infectious virus itself. Since NCI recommends that work with these whole viruses not be prohibited, but rather be performed under containment conditions similar to P3, there is no scientific reason to prohibit recombinant DNA work with these viruses. The "flexibility" clause refers to power of the Director, NIH, to waive prohibitions when the public interest may be served by such action.

(*15*) The Guidelines say at the beginning of Pt. III, "Given below are containment guidelines for permissible experiments. Changes in these levels for specific experiments (or the assignment of levels to experiments not explicitly considered in this section) may be expressly approved by the Director, NIH, on the recommendation of the Recombinant DNA Advisory Committee." Insertion of such language into the guidelines was recommended by the RAC at its Apr. 27-28, 1978, meeting. It recognizes that the classification of experiments given in Pt. III will necessarily be imperfect, as investigators in the future devise new ways to conduct recombinant DNA experiments not currently foreseen and therefore not explicitly considered in the guidelines. Also, new data may become available showing that certain particular experiments currently assigned a particular containment level are, indeed, clearly more (or less) safe than envisioned at this time.

(*16*) See App. C to the guidelines and Part IV of this "Decision Document."

(*17*) In May 1977, a resident of Frederick, Md., brought suit in the U.S. District Court for the District of Columbia to enjoin a proposed risk-assessment experiment which was about to be undertaken in a maximum containment facility (P4) located at the Frederick Cancer Research Center. (*Mack* v. *Califano*, Civil Action No. 77-0916). On February 23, 1978, the court issued a decision refusing to grant the injuction. In so doing, the court observed that the environmental impact statement on the original guidelines constituted a "hard look" at recombinant DNA research performed in accordance with the guidelines. The court further noted that compliance with the guidelines, it appeared, would insure that no recombinant DNA molecules would escape from the carefully controlled laboratory to the environment, and that the guidelines "represent an effort by many scientists to evaluate the hazards and provide safe methods for their control."

The plaintiff appealed (Appeal No. 78-1156), and on Mar. 8, 1978, the Court of Appeals for the District of Columbia upheld the district court decision.

I. Scope of the Guidelines

Review of RAC-Proposed Guidelines

It was the determination of the Recombinant Advisory Committee that advances in knowledge pertaining to recombinant DNA activities in past years warranted significant revisions in the "purpose," "definition," and "prohibition" sections of the NIH guidelines. A comparison of the "purpose" language of the two sets of guidelines[1] reveals that the standards in the PRG-RAC were meant to pertain to recombinant DNA molecules in organisms. The analogues language in the 1976 guidelines addresses recombinant DNA molecules whether or not they are contained within a cell or virus. The rationale for this change is that DNA by itself (commonly referred to as "naked" DNA) is extremely unlikely to be hazardous under experimental conditions, as it is rapidly inactivated in nature.

The definition in the PRG-RAC consisted of two parts: (1) an operational definition of recombinant DNA and (2) a qualification that the guidelines would pertain only to "novel" recombinant DNA's. The operational definition does not differ significantly from that in the original guidelines.

The second part, however, called for the creation of a list of organisms that exchange genetic information in nature, commonly referred to as "non-novel exchangers." Recombinant DNA formed with DNA from such organisms would be exempted from the provisions of the PRG-RAC, with the rationale that there is no justification for requiring containment procedures for the handling of recombinations that occur regularly in nature and are not known to be associated with any special hazards.

The provision of an open-ended listing was recommended rather than issuance or a blanket exemption, because this would allow the RAC and NIH to consider evidence that (1) the putative gene transfers do take place naturally and (2) their exemption from the guidelines is justifiable (see footnote 1 of the PRG-RAC).

Although the PRG-RAC deals with prohibited experiments under Part III, this decision document, for purposes that become apparent below, will consider the definition, exemptions, and prohibitions together under section I.

The "prohibitions" section was called section III-A, "Experiments That Are Not To Be Performed," in both the 1976 guidelines and PRG-RAC. Changes from the 1976 guidelines, proposed in the PRG-RAC, included minor wording changes in items (iii), (iv), and (vi).

The ability to grant exemptions for certain experiments from the "prohibitions" was limited in the 1976 guidelines to only the sixth prohibition (large-scale experiments with recombinant DNA's known to make harmful products). In the PRG-RAC the Director, NIH, is given the authority to grant exceptions from any of the six prohibitions. Such a determination must be based upon the recommendation of the RAC, and weight must be given in the decisionmaking "both to scientific and societal benefits and to potential risks." The rationale for this proposed change was the desire of the RAC not to preclude the possibility of conduction such experiments for some compelling social or scientific reasons—for example, risk-assessment experiments.

The sections of the PRG-RAC dealing with purpose of the guidelines, definition of recombinant DNA, exemptions, and prohibitions evoked a great deal of comment both before and after the December 1977 public hearing. An anylsis of these comments and my decision in response to the issues raised are presented in the following section.

Review of Comments and NIH-Proposed Guidelines

There was considerable discussion at the public hearing over the scope of the guidelines. Some felt that the guidelines were too narrow in their preoccupation with recombinant DNA, as there exist other forms of genetic research capable of producing organisms of unknown potential hazard. It was further suggested that the title of the guidelines be modified to reflect the preoccupation with experiments involving prokaryotes and cells in culture, and that a companion document be released dealing with higher eukaryotes. On the other hand, it was also argued that genetic research has now received attention far beyond its due, and that other matters of experimentation await their turn.

While it is true that other techniques in genetic research, such as cell fusion and chromosome transfer, may result in formation of recombinant molecules, I do not believe at this time that we should mandate or extend the guidelines to these research areas. There are inherent in these techniques a range of natural barriers to the formation of hazardous organisms which apparently afford adequate containment, making unnecessary the issuance of Federal standards. I base this conclusion on the fact that such techniques have been used in the laboratory for decades with no known harmful effects on either the public health or the environment. I should also emphasize that the entire area of laboratory safety is of primary concern to NIH and is the subject of constant review and attention. A description of NIH activities in these areas is presented in the environmental impact assessment.

A commentator suggested that the language be deleted stating that "* * *

[1] The current guidelines as published in the Federal Register, July 7, 1976 (41 FR 27902), and the RAC's proposed revisions (PRG-RAC) as published in the Federal Register, Sept. 27, 1977 (42 FR 49596).

the revised guidelines [have] the intent of erring on the side of caution." While believing that the guidelines are, and should be, deliberately restrictive, I agree with the criticism that scientists should not enter into an activity with the intent of erring. The PRG-NIH now reflects this opinion by deletion of this phrase.

Another commentator suggested that the guidelines should contain language requiring all publications dealing with recombinant DNA activities to include a description of the physical and biological containment procedures used. While the PRG-NIH urges "that all publications dealing with recombinant DNA work include a description of the physical and biological containment procedures employed," NIH is not well advised to dictate to researchers or editors what must be included in a scientific publication.

There were several suggestions that the purpose of the guidelines be more clearly stated and that terms be more precisely defined. I have, therefore, added considerable new material to Part I of the PRG-NIH, renamed "Scope of the Guidelines," and divided it into the following sections, each of which is discussed further below: Purpose; Definition of Recombinant DNA Molecules; General Applicability; Prohibitions; Exemptions; and General Definitions.

Purpose

The introduction to the 1976 guidelines states that "the purpose of these guidelines is to recommend safeguards for research on recombinant DNA molecules." As noted above, to eliminate "naked" recombinant DNA from the guidelines, the PRG-RAC proposed this passage to read that the purpose is to "establish procedures for handling organisms and viruses containing recombinant DNA molecules."

This proposed revision would have had the effect of removing from coverage by the guidelines certain experiments which are prohibited by the 1976 guidelines—for example, deliberate formation of naked recombinant DNA containing genes for the biosynthesis of potent toxins. I have decided to resolve this issue conservatively. The language in the PRG-NIH, therefore, clearly states that the guidelines are intended to pertain to the construction and handling of naked recombinant DNA molecules as well as of organisms and viruses containing such molecules.

General applicability

Many commentators urged that a statement of general applicability of the guidelines be included in an early part. The issues relate to (1) the applicability of the guidelines to non-NIH funded research with recombinant DNA at institutions receiving NIH funds for this purpose, (2) the applicability of the guidelines to NIH-supported recombinant DNA research conducted in foreign countries, and (3) the location of responsibility for insuring compliance with the guidelines. Therefore, a section entitled "General Applicability" now appears after the "Purpose" section in Part I of the PRG-NIH.

The existence of guidelines for recombinant DNA research assumes their general application. Partial adherence within an institution would defeat the purpose of extending maximal protection to the community. Thus, it would be inconsistent for NIH to provide funds for biomedical research activities to an institution that did not meet the standards of the guidelines in all of its recombinant DNA research, regardless of the source of funding. This principle is now stated explicity in the PRG-NIH, and we intend to consider withholding NIH funds as a sanction against violation.

Rules must be established for the conduct of recombinant DNA activities funded by NIH in other countries. Generally, the requirements in force in those countries shall apply. A memorandum of understanding and agreement (MUA) must still be filed with NIH, indicating specifically which guidelines will govern the activities; and NIH reserves the right to withhold funding if the safety practices to be employed are not comparable to the NIH guidelines. An explicit statement about this has been inserted in the PRG-NIH.

Part IV of the PRG-NIH describes the responsibilities of all individuals and organizational entities involved in the conduct and review of a recombinant DNA activity. Two years of experience with administering the NIH guidelines has indicated that the ultimate responsibility for insuring compliance must be borne by the institution where the research is being done. This implies some discretion under well-defined limits for interpretation of common standards, and imposes a requirement for local expertise other than the investigator's. Accordingly, Part I of the PRG-NIH now requires that an individual receiving NIH support for recombinant DNA research be associated with an institution that is willing and able to accept the responsibilities and conditions of local governance, described more fully in Part IV of the PRG-NIH.

Definition of recombinant DNA molecules

It became apparent from the comments received that the PRG-RAC definition was inadequate in that it did not address the handling of recombinant DNA molecules containing segments of chemically synthesized DNA. I have decided that the most effective way to achieve this objective is simply to include "natural or synthetic DNA" in the definition of a recombinant DNA molecule, and this has been inserted in the PRG-NIH definition. A new section, therefore, has also been added to Part III of the PRG-NIH giving containment levels for work with recombinant DNA molecules containing synthetic DNA.

I have also revised what I perceived to be an ambiguity in the PRG-RAC definition by including within the PRG-NIH definition language explicitly stating that DNA molecules which result from the replication of recombinant DNA molecules are subject to the safety provisions of the guidelines.

Finally, no other provision of the PRG-RAC definition evoked as much comment as did the wording to exclude "non-novel" recombinant DNA from the standards. The ambiguity of such phrases as "known to exchange chromosomal DNA" and "by natural physiological processess" was strongly noted, and I agree with the commentators that we must strive for a greater degree of clarity and objectivity. Thus, it has been decided to eliminate in the PRG-NIH the two conditions cited above as criteria for exemption from the guidelines. Staff discussions of the public comments made it clear that inclusion of exemption provisions within the definition itself was not desirable. Several attempts at appropriate language did not bear careful scrutiny.

Given this situation, and also my opinion that certain categories of recombinant DNA experiments are indeed so apparently free of causing harm that they should not come under the guidelines, it was my decision to remove the criterion of "novelty" from the definition and use it as a basis for the development of a new section entitled "Exemptions."

Exemptions

The nature of the public comments on the PRG-RAC exclusion of nonnovel exchangers can be divided into categories—those that pertain to the proposed standards and those to the proposed process.

The standards proposed by the PRG-RAC were that novel recombinant DNA's are those consisting of "segments of any DNA from different species not known to exchange chromosomal DNA by natural physiological processes * * * In general recombinant DNA molecules * * * will not be considered novel when all the components are derived from genomes known to replicate within the organism used to propagate the recombinant DNA." This is qualified, however, by a footnote stating that the "recombinant DNA formed between segments of eu-

karyotic viral DNA and any eukaryotic DNA * * * shall not be excluded * * * until such time as there is more information about the extent of naturally occurring recombinational events between these DNAs."

The public comments on these standards raised the following issues:

- That safety rather than novelty should be the criterion for exclusion; that is, any recombinant DNA molecule that poses a threat to the public health or the environment should be covered by the guidelines regardless of whether the molecule is a novel one.
- Others argued that the proper criterion should not be safety but rather whether the potential hazard of the recombinant DNA molecules differs significantly in degree or in kind from those found in nature or from biohazards that are successfully handled by conventional methods.
- That there is a question of quantification that goes beyond novelty; that is, a recombinational event that occurs very rarely in nature can be mimicked more frequently in the laboratory.
- That the PRG-RAC was ambiguous in describing the criteria to be used in judging novelty.
- That the list of nonnovel exchangers should not be limited to the exchange of chromosomal DNA, but should also include plasmid DNA exchange.
- That the list should not be drawn broadly at the species level, but should deal with exchange at subspecies levels.
- That footnote 1 of the PRG-RAC unjustly discrimated against natural recombinants involving eukaryotic viral DNA and other eukaryotic DNA. Others urged that this footnote be expanded to ensure that recombinants involving pathogenic bacteria not appear on the list.
- That experiments classified as P1+EK1 be exempted from the guidelines. Apparently harmless experiments do not warrant the administrative burden that accompanies inclusion within the guidelines.
- That it was unclear whether the PRG-RAC definition woud permit "self-cloning" experiments (such as the cloning of *B. subtilis* genes in *B. subtilis*).

It proved impossible to reconcile these differences of opinion in the definition itself, but in my opinion the "Exemptions" section of the PRG-NIH as drafted does so successfully. This section was drafted by NIH staff in conjunction with a working group of the RAC; it was then modified slightly and endorsed by the full RAC at its meeting on April 27-28, 1978, and subsequently modified slightly for clarity by NIH staff. Before proceeding to a discussion of these exemptions, however, I want to emphasize that no provision in this section may be cited to exempt from the guidelines an activity listed in the "Prohibitions" section.

The first exemption concerns recombinant DNA molecules that are not in organisms or viruses. This is in recognition that "naked" DNA, which is rapidly inactivated in nature, is extremely unlikely to be hazardous under experimental conditions. To guard against the remote possibility, however, that potentially harmful naked recombinant DNA will be incorporated into an organism, the handling of certain naked recombinant DNA molecules described in the "Prohibitions" section remains prohibited. It should also be noted that the concept of extremely low hazard of naked recombinant DNA was included in the PRG-RAC in the section on "Handling Recombinant DNA Molecules" at the end of part III. This language, I believe, is more appropriately presented under the "Exemptions" section.

The second exemption pertains to recombinant DNA molecules consisting entirely of DNA segments from a single nonchromosomal or viral source. This statement clarifies a category of "self-cloning" experiments that are considered safe enough to be excluded from the guidelines. This is a concept which the RAC tried to convey in the PRG-RAC definition by use of the phrase "different genomes," but which some commentators found ambiguous.

The third exemption concerns "self-cloning." It exempts from the guidelines recombinant DNA molecules made entirely from the DNA of a single organism including the plasmids, viruses, mitochondria, or chloroplasts indigenous to (i.e., found in nature in) that organism, when propagated only in that organism (or a closely related strain of the same species). This partially responds to the suggestion made by many commentators that experiments previously classified as P1+EK1 be excluded from the guidelines. It also covers some of the cases the RAC was including in the concepts of "novelty" and "different genomes." This exemption, however, does not include recombinant DNA molecules formed between viral DNA and eukaryotic host DNA. In this regard it is analogous to footnote 1 of the PRG-RAC.

The fourth exemption covers "certain specified recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes." In this case a list is prepared and periodically revised by the Director, NIH, on the recommendation of the RAC, after appropriate notice and opportunity for public comment. This list is analogous to the list of "nonnovel exchangers" proposed in the PRG-RAC.

The initial entries on the list specified under exemption I-E-4 are given in appendix A to the guidelines. Any recombinant DNA molecules composed entirely of DNA segments coming from organisms listed in appendix A, would be exempt from the PRG-NIH under exemption I-E-4. The inclusion of the particular organisms listed in appendix A was recommended by the RAC at its meeting on April 27-28, 1978. (For further discussion of this list see appendix D to the accompanying Environmental Impact Assessment.)

The fifth exemption allows the Director, NIH, on the recommendation of the RAC, after appropriate notice and opportunity for public comment, to exempt other classes of recombinant DNA molecules if he finds that "they do not present a significant risk to health or the environment." The exemption of classes of experiments that do "not present a significant risk to health or the environment" is the language used in proposed legislation (H.R. 11192), recently reported out of the committee on Interstate and Foreign Commmerce and the Committee on Science and Technology of the U.S. House of Representatives.

In addition to comments pertaining to the standards for exemption in the PRG-RAC, the following comments were directed toward the processes whereby exemptions would be made:

- Rather than compile a list of nonnovel exchangers exempt from the guidelines, the burden of proof should be on the Director, NIH, to compile a list of novel exchangers which are subject to the guidelines.
- The procedures and critieria used in the development of the list should be explained thoroughly, and adequate opportunity should be given for public review and comment.
- Before being placed on the list, all the data pertaining to the application should be available for public review.

In response to these comments, the PRG-NIH specifies that for exemption I-E-4 and I-E-5—the two exemptions which involve the development of "lists"—these lists will be prepared by the Director, NIH, on the advice of the RAC, *after appropriate notice and opportunity for public comment.* Publication of the PRG-NIH includes appendix A giving an initial proposed list for exemption I-E-4. As part of the public comment which I am soliciting on the entire PRG-NIH, I include appendix A. In the future, no additions will be made to appendix A, nor will any items be listed as exemptions under exemption I-E-5, without ap-

propriate notice and opportunity for public comment.

Prohibitions

Two changes in this section have been initiated to make it more compatible with the new "Definition" and "Exemptions" sections. The first was to transfer this section from part III of the guidelines to part I. This is again to emphasize that the exemptions are not applicable to the six activities listed as being prohibited. The second was to drop all references to novel recombinant DNA's and natural genetic exchange. My other actions were based upon the following comments:

● There was general endorsement of the provision in this section which grants to the Director, NIH, upon the recommendation of the RAC, the authority to waive any of the prohibitions. The widespread support for this authority reflects the realization that many important risk-assessments experiments may not be able to proceed otherwise. NIH is now supporting and will continue to support experiments that will yield knowledge contributing to a better understanding of the nature of potential risks of recombinant DNA. This section has been expended in the PRG-NIH to indicate that if any experiments are excepted from the prohibitions, they will "at that time be assigned appropriate levels of physical and biological containment."

● It was urged that the advice of other Government agencies, such as the Environmental Protection Agency (EPA) and the Occupational Safety and Health Administration (OSHA), should be sought when the Director, NIH, considers invoking this waiver authority. The Federal Interagency Committee on Recombinant DNA Research provides for coordination of policies in this area. EPA and OSHA are represented on the Committee. The advice of relevant research and regulatory agencies will continue to be sought when appropriate.

● It was suggested that the RAC as presently constituted should not be the sole advisory body because societal as well as scientific considerations must enter into the waiver decision. As explained in greater detail in part IV of this document, the membership of the RAC will be broadened modestly as needed for expertise, but provisions for public notice and opportunity to comment, and other appropriate adminstrative practices, can be used to ensure adequate public input when the issues warrant.

● It was suggested that an Environmental Impact Assessment or Statement should accompany each waiver. My waiver decisions will include a careful consideration of the potential environmental impact, and certain decisions may be accompanied by a formal assessment or statement. This must be determined on a case-by-case basis.

● It was suggested that waiver of the prohibition on the large-scale use of culture containing recombinant DNA's be issued on the basis of industry's experience in dealing with such cultures. While such experience will surely be weighed in the decisionmaking, I believe that it should not be the sole criterion for granting such a waiver.

● Agricultural scientists noted the importance to their research community of being allowed eventually to release organisms containing recombinant DNA into the environment. When the original guidelines were presented to me in draft form in 1976, the release of organisms containing recombinant DNA molecules into the environment was to be allowed if a series of controlled tests had been done to leave no reasonable doubt of safety. At that time I rejected this waiver provision because of the limited scientific evidence available that any of the potential benefits from such a release were near realization.

The prohibition of deliberate release into the environment of recombinant DNA-containing organisms can be waived if all of the requirements for a waiver are met (and if the requirements of the National Environmental Policy Act are considered). Given the limited experience of NIH in agricultural research, the U.S. Department of Agriculture would be deeply involved in this process. I have given written notice of this opinion to the appropriate officials of the USDA.

● The Standing Advisory Committee on Recombinant DNA Research of the European Molecular Biology Organization (EMBO) has noted that the list of pathogenic organisms under prohibition I-D-1, especially those in class 5, may not be appropriate for all European countries, and that "the decision as to which pathogenic orgamisms should be classified as too dangerous to use must be the responsibility of national or regional authorities." In response to this a footnote could be added to the guidelines stating that prohibition I-D-1 relates only to research in the United States. I have decided, however, not to include such a footnote, because these guidelines are directed to NIH grantees and contractors, almost all within this country. In other countries, different criteria may govern.

● A final change in the PRG-NIH relates to prohibition I-D-1. As discussed below in this document in part III, considerable changes have been made in the sections dealing with the use of viral DNA in recombinant DNA experiments. The history leading to these changes, including the report of the "Ascot" workshop (appearing as appendix E to the accompanying Environmental Impact Assessment) and the report of the working group held on April 6-7, 1978 (appearing as appendix F to the accompanying Environmental Impact Assessment), are discussed in detail in part III of this document under the heading "Recombinant DNA Experiments Involving Viral DNA."

One of the Working Group's recommendations, arising out of the "Ascot" report and endorsed by the RAC at its April 27-28, 1978, meeting and endorsed by me, is that the previous prohibition on the use in recombinant DNA experiments of Vesicular Stomatitis Virus (VSV) and of oncogenic viruses classified by the National Cancer Institute (NCI) as "moderate risk" should be lifted; instead, use of these viruses should be permitted under containment conditions to be specified in part III of the guidelines. The reasoning behind this is that recombinant DNA experiments with pieces of these viruses cloned in *E. coli* K-12 pose no more risk, and actually appear to pose clearly less risk, than work with the whole infectious virus itself. Since the Center for Disease Control (CDC) and NCI recommend that work with these whole viruses not be prohibited, but rather be performed under containment conditions similar to P3, there is no scientific reason to prohibit recombinant DNA work with these viruses.

Therefore, prohibition I-D-1 in the PRG-NIH no longer prohibits the use of VSV or oncogenic viruses classified by NCI as moderate risk; containment conditions for their use are specified in part III of the guidelines.

General Definitions

In response to commentators' suggestions that terms be more precisely defined, I have added a new section to the PRG-NIH with such definitions. Many of these terms are further discussed in part IV of PRG-NIH.

In summary, part I of the PRG-NIH has been extensively modified from that proposed in the PRG-RAC. In an effort to be responsive to the suggestions of commentators and to make the guidelines more comprehensible, the definition of recombinant DNA molecules has been simplified and clarified, the "Prohibitions" section has been transferred from part III to part I, and new sections have been added to part I including "Exemptions." part I, now entitled "Scope of the Guidelines." is composed of the following sections:

● Purpose

● Definition of Recombinant DNA Molecules

- General Applicability
- Prohibitions
- Exemptions
- General Definitions

It should be noted that the Prohibitions appear before the Exemptions. This will again emphasize the fact that the latter provisions cannot be used to claim relief from the former.

II. Containment

The object of these revised guidelines is to insure that experimental DNA recombination will have no ill effects on the researchers, on the general public, or on the environment. The essence of their construction, as in the case of the 1976 Guidelines, is subdivision of potential experiments by class, and assignment to these of certain procedures for containment.

Containment is both physical and biological. *Physical* containment involves the isolation of the research by procedures that have evolved over many years of experience in laboratories studying infectious micro-organisms. P1 containment—the first physical containment level—is that used in most routine bacteriology laboratories. P2 and P3 afford increasing isolation of the research from the environment. P4 represents the most extreme measures used for containing virulent pathogens, and permits no escape of contaminated air, wastes, or untreated materials. *Biological* containment is the use of biological agents that are crippled by mutation so as to be incapable of surviving under natural conditions.

PHYSICAL CONTAINMENT

Review of RAC-Proposed Guidelines

Two major changes were proposed in the physical containment section of the PRG-RAC. One deals with the organization of the section; the other incorporates into the PRG-RAC the philosophy and guidance of the report of the NIH European Molecular Biology Organization (EMBO) Workshop on parameters of physical containment.[2]

Physical containment requirements for each P level have been organized under the topic headings *Laboratory Practices, Containment Equipment,* and *Special Laboratory Design.* This was done to emphasize the importance of laboratory practices and containment equipment in achieving the desired safety objective.

Other revisions contained in the "Physical Containment" section reflect a conscious effort to encourage international uniformity with respect to recombinant DNA guidelines. This has been achieved by revising the containment descriptions so that they are consistent with the guidance provided in the NIH/EMBO report. In addition, some statements have been rewritten and others added in order to clarify the basic requirements for each level of containment. The most significant clarifications were made in the areas on containment equipment and special facility design. The revisions, however, have not resulted in changing the purpose or intent of the physical containment descriptions in the 1976 Guidelines.

Two specific additions to the Guidelines that originated from the NIH/EMBO report are particularly notable. The first is that Tables I and II have been added to the P3 and P4 sections, respectively. These tables show combinations of safeguards that provide similar protection. The combinations are dependent on the level of biological containment. This approach allows flexibility in selecting containment equipment for a particular study without compromising safety.

The second specific addition is the inclusion of laboratory design criteria for an area in which personnel wear positive-pressure suits ventilated by life-support systems. This added approach provides a level of physical containment equivalent to that afforded by the glove-box cabinet requirement at the P4 level.

Other important changes are summarized below:

- Certain specific microbiological practices are mandated at the P1 level in the PRG-RAC (whereas in the 1976 Guidelines they were merely encouraged);
- At the P2 level, prohibitions against eating, drinking, smoking, and storage of foods have been extended from the work area to the entire laboratory;
- The universal biohazard sign is now required at the P2 level. Use of these signs has been extended to equipment such as freezers and refrigerators in which organisms containing recombinant DNA molecules are stored;
- Access procedures in controlled areas adjacent to P3 laboratories have been specified;
- Installation of foot-, elbow-, or automatically-operated facilities for washing hands is now required in P3-level laboratories;
- Specific guidance on containment equipment appropriate for laboratory animals has been added to the P3 and P4 sections;
- The labeling requirements for shipment of etiologic agents now apply to all organisms containing recombinant DNA molecules. Thus, the Center for Disease Control, U.S. Public Health Service, must be notified in the event of any accidental breakage during shipment. Also, agents requiring P4 containment must be packaged according to strict Federal standards and be shipped by registered mail or an equivalent system that provides for notifying the shipper upon delivery.

I have carefully reviewed the recommendations of the PRG-RAC relating to physical containment and propose to adopt them with certain modifications. The modifications, based on issues raised by the Director's Advisory Committee and public commentators, are discussed below.

Review of comments and NIH-proposed guidelines

As reported in the "Decision Document" which accompanied the release of the 1976 guidelines, comments on the containment provisions of the original Guidelines were directed to the definitions of both physical and biological containment and to the safety and effectiveness of the prescribed levels. Several commentators at that time found the concept of physical containment imprecise and subject to human error. Others questioned the concept of biological containment in terms of its safety and purported effectiveness in averting potential hazards. The commentators were divided on which method of containment would provide the most effective and safe system.

Several suggested that each of the physical levels be explained more fully. The physical containment section of the 1976 guidelines—and now of the PRG-NIH—is directly responsive to many of these commentators. In addition, the PRG-NIH takes into account the more recent comments related to standards for physical and biological containment. Commentators on the PRG-RAC have expressed particular concern over (1) the flexibility which allows various combinations of containment safeguards, (2) the design of containment systems, and (3) the adequacy of training in laboratory safety practices. The Standing Advisory Committee on Recombinant DNA Research of the European Molecular Biology Organization (EMBO) made a number of recommendations that NIH has considered, and public commentators have proffered additional suggestions relating to specific levels of physical containment and to shipment of recombinant DNA materials. These are examined below.

Concept of "Flexibility." Some commentators have expressed concern over the flexibility provided in Tables I and II that allows various combinations of containment safeguards. For

[2] The "Report of the NIH/EMBO Workshop (Parameters of Physical Containment)" may be obtained from the Office of Research Safety, National Cancer Institute, Room 3E47, Building 13, National Institutes of Health, 9000 Rockville Pike, Bethesda, Md. 20014.

example, some feel that work in a P3 facility conveys a desirable sense of hazard, whereas a reduction to the P2 level will promote an undesirable relaxation of vigilance. it has also been suggested that an increase in the options increases the difficulty of control and implementation of the guidelines. Some commentators object to specific options provided at the P3 and P4 levels. NIH has been urged to include a better explanation of the rationale for this flexibility.

Indeed, the calculus of switching physical and biological containment levels has been questioned. Does an increase in biological containment from EK1 to EK2 truly compensate a reduction in physical containment from P3 to P2?

The scale of either form of containment from least to greatest is not necessarily linear, and substitutions are only roughly approximate. Nevertheless, there are some numerical bases for comparison.

For example, a class III biological safety cabinet is required at the P4 level (if a positive pressure suit is not used); whereas at P3, one can work in an open-front biological safety cabinet. The class III cabinet is virtually an absolute containment system. It is certified gas-tight when tested under positive pressure. It is operated under negative pressure to gain optimum safety. It provides at least a 10,000- to 100,000-fold increase in safety over that provided by a Class I or II cabinet, which is required at the P3 level.

The relative safety of these two containment cabinets is based on the efficiency of their exhaust-air treatment systems. The exhaust-air treatment for the class III cabinet is provided by two HEPA filters installed in series. This arrangement gives a containment efficiency of at least 99.99 percent. The exhaust-air treatment for class I and II cabinets, with only one HEPA filter, provides a containment efficiency of 99.99 percent. The potential for escape of microorganisms across the open front of the class I and II cabinets is similar to that for escape through the exhaust-air treatment system under operating conditions. These cabinets must meet a performance criterion which permits fewer than 20 microorganisms to escape through the open front when 1×10^8 (100,000,000) to 8×10^8 (800,000,000) microorganisms are experimentally released within the cabinet. The degree of protection provided by the class I or II cabinets is equivalent to the increase in safety at the P3 level over that provided at the P1 level which allows open-bench operations.

The symbol HV (Host-Vector) is used in the PRG-NIH to designate biological containment systems encompassing the present EK systems. HV2 is defined in terms of a probability of escape of recombinant DNA of less than 1 in 10^8 (1 in 100,000,000). in considering "equivalency" between P and EK levels, it is recognized that the two systems are conceptually different. Biological safety cabinets are designed primarily for the protection of the laboratory worker, and all physical containment protection stops at the walls of the laboratory. Biological containment continues to operate even were an organism to escape from the laboratory.

The flexibility allowed in alternate P and HV levels is carefully explained in the text of the PRG-NIH, and the investigator must follow the explicit requirements set forth in Part III of the proposed guidelines and Tables I and II.

Redundancy. A question has been raised concerning redundancy in the safety systems to insure that alternate systems will come into play in case of an emergency—for example, power failures or major accidents. The concept of redundancy is inherent in the design of the containment systems used in recombinant DNA research. Redundancy, however, is provided by standby systems, but rather by design features and operational requirements of the safety systems used. For example, primary containment at the P4 level is provided by the gas-tight class III cabinet system. These cabinets are also maintained under negative air pressure, which would provide protection against the release of microrganisms in the event that a glove were to rupture or a leak to develop. Similarly, the physical isolation of the class III cabinet would not be compromised in the event of a power failure. However, since the redundant protection provided by the negative pressure would be compromised, personnel would be instructed to stop work immediately during the power interruption. Another example is the requirement that the exhaust and supply fans for P4 facilities be interlocked. This assures that in the event of failure of the exhaust fan, the supply fan will automatically shut down, preventing the pressurization of the laboratory environment. As with the class III cabinet example, personnel would stop their work because of the loss of secondary protection provided by the ventilation systems. Operational procedures, therefore, become an important element in assuring safety in the event of any system failure.

Institutions are required to devise emergency plans to handle possible problems. In response to recommendations of the Environmental Protection Agency Study Group on Recombinant DNA and to concerns raised by commentators, NIH has stipulated more clearly (in the supplement to the PRG-NIH entitled, "Laboratory Safety Monograph") certain elements in these emergency plans. Moreover, NIH staff have recently met with representatives of the center for Disease Control (CDC) to establish a mechanism for providing advice, consultation, or assistance, if necessary, in case of an emergency, such as an accident in the laboratory.

Laboratory safety. A number of commentators felt that the PRG-RAC was vague in regard to the training in safety of researchers, students, and janitors. It was urged that specific curricula be developed and that a requirement for certification of training be stipulated in the guidelines (a recommendation also made by the EPA Study Group on Recombinant DNA). It has been suggested, further, that NIH develop curricula for training.

At the present time, NIH has a contract with the American Society for Microbiology (ASM) to develop minimum standards for training participants in recombinant DNA research. The ASM Working Panel will consider what standards of training in microbiologic techniques are appropriate for the conduct of experiment requiring P1 through P3 containment conditions. The Panel will solicit views from the scientific community to develop minimum requirements for training. The Panel's report will be made available to the IBC's and investigators to set standards for all who participate in this research. In view of these developments, formal certification requirements by NIH are considered premature.

Other commentators stressed the need for more stringent measures in regard to safe practices. In particular, these commentators urged regular monitoring of laboratory facilities, preferably at all P levels. This would include monitoring of microbiological practices, serological monitoring, and CDC review of incidence of infections. It was also suggested that regular inspections be performed by individuals not associated with the institution (to preclude conflict of interest); that the guidelines require a member of the work force to be represented on the institutional biosafety committee; and that penalties (other than cutoff of funds) be imposed on violators as a deterrent. I have accepted many of these proposals; the specific NIH actions in regard to them are discussed in Part IV of this document.

Appendix D, "Supplementary Information on Physical Containment," was added to the 1976 guidelines in response to numerous requests for greater specificity in describing containment requirements. Commentators noted the absence of this document from the PRG-RAC and urged that it be retained and further expanded. Ac-

cordingly, a special committee of safety and health experts was convened by W. Emmett Barkley, Ph. D., Director of the Office of Research Safety, National Cancer Institute, to review and revise this supplementary information. Several sections have been extensively rewritten, and new sections have been added on evaluation methods for P3 facilities, certification procedures for P4 facilities, certification of biological safety cabinets, emergency control procedures, medical surveillance programs, and other topics. This document is separately available as "Laboratory Safety Monograph—A Supplement to the NIH Guidelines for Recombinant DNA Research."

Other comments. A number of additional comments have been received from public commentators relating to proposed actions at specific levels of physical containment.

It has been suggested that certain requirements at the P1 level remain "permissive" rather than be changed to "mandatory"; i.e., that the language in the PRG-NIH read "should" rather than "shall." NIH considers this inconsistent with the stated principle of specifying requirements, and has therefore mandated adherence to these good microbiological practices.

The EMBO Standing Advisory Committee on Recombinant DNA Research has recommended that simple air exhaust cabinets be used at the P1 level when there is likelihood of producing large amounts of aerosols. In the view of NIH such cabinets are unnecessary, as the agents used at this level would not create aerosols hazardous to laboratory workers.

A recommendation has been received from the EMBO Standing Advisory Committee on Recombinant DNA Research to reclassify P2 with a class III cabinet as equivalent to P3 specifications. While this option was permitted in the 1976 guidelines, it is no longer considered practical. The cost of fabricating and installing class III cabinets would far exceed the cost of installing a new exhaust-air system for the laboratory. It is considered more cost-effective and desirable to convert P2 laboratories into P3 laboratories. The elimination of the 1976 option should be viewed as an encouragement to upgrade laboratories.

It has been observed that many class II safety cabinets do not meet accepted standards. A recommendation has been made that the local IBC be authorized to certify these cabinets, and that such a requirement be included in the guidelines. It should be noted that the guidelines already authorize IBC's to certify safety practices and procedures; however, to respond more directly to the above suggestion, a special section on certification of biological safety cabinets has been included in the supplement to the PRG-NIH entitled "Laboratory Safety Monograph."

The EMBO Standing Advisory Committee on Recombinant DNA Research observes that in the case of a P3 facility, the proposed revisions do not speak to precautions against the contamination of the main water supplies by laboratory water systems. It is noted that building codes and laboratory design standards require that precautionary measures be taken to separate potable water systems from laboratory process water. Additional precautions have been required at the P4 level. Standard design practice is felt to be appropriate at the P3 level.

Some commentators have pointed out that the PRG-RAC did not require an autoclave in the P3 laboratory itself, but only within the building. The 1976 guidelines require that for P3 laboratories an autoclave be available "within the building and preferably within the controlled laboratory area." Some believe an autoclave in the P3 laboratory should be required. One commentator felt that the autoclave should be "as close as possible" to the controlled area of the P3 laboratory, not merely available in the same building. He pointed out that from an operational point of view, the closer the autoclave can be to the solid waste, the better. This is especially true in the larger medical research complexes, where transport of wastes from the laboratory to the autoclave might involve passage "via some rather sensitive patient areas of the institution." He prefers that the autoclave be located either in the controlled area or as close to it as possible, with such explicit language in the guidelines. The language in the 1976 guidelines stating that in a P3 laboratory "an autoclave shall be available within the building and preferably within the controlled laboratory area" has been reinserted in the PRG-NIH. However, an absolute requirement that the autoclave must be within the controlled area is not considered appropriate, since contaminated materials can be safely transported. Such a requirement would exclude the use of autoclaves in waste staging areas that have been conveniently sited to support an entire facility.

The PRG-RAC states that P4 work can be done in either (1) a class III cabinet system or (2) a class I or class II cabinet system in a special area where all personnel wear one-piece, positive-pressure suits. Some investigators apparently prefer use of pressure suits over work in the class III cabinets. NIH believes that the suits are especially useful in working with experimental animals in a P4 facility or with large amounts of material. At present, however, most recombinant DNA studies are handled more practically in a class III without need for a suit.

In 1976, several commentators advocated that NIH arrange for sharing of P4 facilities, both by investigators from the NIH intramural program and from institutions supported through NIH awards. In response to these suggestions and those of recent commentators, we have arranged to make our recently established P4 facilities at the Frederick Cancer Research Center (Fort Detrick) available to outside scientists.

Shipment. Some commentators have urged that stricter controls be required on shipping recombinant DNA molecules in or out of the country. It has been recommended, for example, that shipping procedures differentiate between types of substances being transported. We wish to emphasize that requirements for shipping organisms that contain recombinant DNA molecules are consistent with relevant Public Health Service, Department of Transportation, and Civil Aeronautics Board regulations, and are also in compliance with the World Health Organization recommendations on the international shipment of biologic agents. It should be noted that organisms containing recombinant DNA molecules all require the same containment conditions as for the most hazardous known agents.

The EMBO Standing Advisory Committee on Recombinant DNA Research recommends that before a shipment is made, the recipients of organisms containing recombinant DNA molecules should affirm to the donors that they are following the safety standards and practices of their country. NIH considers this a sound recommendation and requires the following (as stated in the NIH Guide for grants and contracts):

All memoranda of understanding and agreement (MUA's) submitted with competing and noncompeting applications involving recombinant DNA research must indicate that the principal investigator (program director, fellow, or candidate) agrees to comply with the NIH Guidelines and other specific NIH instructions pertaining to the proposed project. Included in the provisions are the following pertaining to shipment or transfer or recombinant DNA materials:

A. Prior to shipment or transfer of recombinant DNA materials to other Federally funded investigators within the United States, the sending laboratory shall obtain a letter from the requesting laboratory stating that:

1. Research involving recombinant DNA molecules shall be conducted in compliance with the NIH Guidelines and other NIH instructions, and that the requesting laboratory shall not transfer the recombinant DNA materials to other laboratories;

2. The requesting laboratory has been reviewed by its Institutional Biosafety Com-

mittee which has certified that facilities, procedures, and the training and expertise of the personnel involved are adequate;

3. An approve MUA with a certification is on file with the funding agency of the requesting laboratory;

4. A copy of this letter is on file with the requesting laboratory's Institutional Biohazards Committee.

B. Prior to shipment or transfer of recombinant DNA materials to non-Federally funded investigators or institutions within the United States, the sending laboratory shall obtain a letter from the requesting laboratory stating items 1, 2, and 4 under A above.

C. Prior to international shipment of recombinant DNA materials, the sending laboratory shall obtain a statement from the requesting laboratory stating that research involving recombinant DNA molecules shall be conducted in accordance with the containment levels specified by the NIH Guidelines, or applicable national guidelines if such have been adopted by the country in which the research is to be conducted, and that the requesting laboratory shall not transfer the recombinant DNA material to other laboratories.

D. The sending laboratory shall maintain a record of all shipments of recombinant DNA materials and shall provide NIH with a complete list of such shipments in the annual progess report for NIH grants and contracts.

Mouth-pipetting at the P1 level. Both the 1976 guidelines and the PRG-RAC prohibit mouth-pipetting at the P2, P3, and P4 levels. For the P1 level, however, they state, "Although pipetting by mouth is permitted, it is preferable that mechanical pipetting devices be used. When pipetting by mouth, cotton-plugged pipettes shall be employed." A number of commentators have urged that mouth-pipetting be prohibited at the P1 level of physical containment. This is strongly endorsed by NIH safety experts, who point out that this is an important safety feature, and that efficient new mechanical pipetting aids should not greatly hamper researchers. Also, the EMBO Standing Advisory Committee on Recombinant DNA Research "believes that mouth pipetting should be prohibited in the P1 laboratory, as it is prohibited in P2-P4 laboratories." In addition, the Working Group of American virologists which met on April 6-7, 1978, to review the report of the U.S.-EMBO Workshop to Assess Risks for Recombinant Experiments Involving the Genomes of Animal, Plant, and Insect Viruses[1] wrote the following in their report:

> In its deliberations, the Working Group was impressed with the safeguards afforded by a ban on mouth pipetting for recombinant DNA experiments involving *E. coli* K-12 host-vectors. The group felt that the only plausible way *E. coli* K-12 could gain entry into laboratory workers was by oral ingestion. The analysis contained in the U.S.-EMBO Report was predicated on the remote possibility that *E. coli* K-12, containing eukaryotic viral DNA, would be swallowed and the viral DNA insert would be delivered to a tissue in the body which ordinarily would be inaccessible to the virus. A prohibition of mouth pipetting would clearly prevent this sequence of events from even beginning. The Working Group therefore recommended that no mouth pipetting be allowed at *any* level of physical containment (including P1) when working with *E. coli* K-12.

On the other hand, when I requested that the RAC, at their April 27-28, 1978, meeting reconsider whether mouth pipetting should not be banned at the P1 level, it was their consensus that many experiments classified as P1 need not include a ban on mouth-pipetting, and that therefore P1 in general should not be redefined. Instead, they recommended that only certain classes of P1 experiments be designated as requiring no mouth-pipetting.

In resolving this issue, I have decided to adopt the conservative position and ban mouth-pipetting. Accordingly, language has been inserted in the PRG-NIH saying that at the P1 level, "Mechanical pipetting devices shall be used; pipetting by mouth is prohibited." Since mouth-pipetting had already been banned at the P2-P-4 levels, this means that it is now banned for all experiments covered by these guidelines.

BIOLOGICAL CONTAINMENT

Review of RAC-proposed guidelines

Experiments on recombinant DNA's by their very nature lend themselves to applications of highly specific biological barriers as a means of containment. In fact, there are natural barriers that limit either the infectivity of a vector or vehicle (plasmid or virus) to specific hosts, or its dissemination and survival in the environment. Both the vectors whereby DNA is transferred to the recipient host and the host cells wherein it replicates can be designed genetically to decrease by many orders of magnitude the probability of dissemination of recombinant DNA outside the laboratory.

The proposed revised guidelines describe the categories of hosts and vectors to be used in minimizing the spread of organisms containing recombinant DNA. The PRG-RAC differs in some respects from the 1976 guidelines as a result of certain changes in definitions of HV systems and in the requirements at specific HV levels (notably HV3). A new section has been added on certification of host-vector systems.

Definitions of host-vector systems. A new nomenclature—HV1, HV2, and HV3—has been developed to incorporate a variety of hosts and vectors into the framework initially established for *E. coli* K-12. In particular, the PRG-RAC provides criteria for HV1 systems other than *E. coli* K-12. In the 1976 guidelines, cloning systems other than *E. coli* K-12 were to be considered only if superior to *E. coli* K-12 in containment properties; but it is now recognized that many useful experiments can only be conducted using HV systems other than those based on *E. coli* K-12, and that such experiments should be permitted so long as the proposed HV system provides equivalent biological containment. The new HV1 criteria provide a structure for approval of systems that meet these requirements.[3]

HV2 systems. AT the HV2 level of containment, there are no substantive changes comparing the 1976 guidelines with the PRG RAC. However, the RAC, on June 23, 1977—the same day it approved the PRG-RAC—also adopted unanimously "Instructions to Investigators Concerning Data To Be Submitted on Host-Plasmid Systems Proposed for EK2 Certification." Although not officially part of the PRG-RAC, these instructions set forth criteria that any putative EK2 host-vector systems must meet before recommendation by the RAC for certification. The RAC applied these criteria in reviewing new systems (pBR322 and pBR313 in x1776) at the June 23, 1977, meeting, and will do so for all future submissions. It was made clear at the meeting that these criteria are definitely more stringent than previous ones, and this greater stringency means that EK2 host-vector systems approved now and to be approved in the future are even safer than those approved previously.

Requirements for HV3 systems. These have been made more stringent in the PRG-RAC than the corresponding requirements for EK3 in the 1976 guidelines. The PRG-RAC requires that the vector be dependent on its propagation host or be highly defective in mobilizability. "Reversion to host-independence must be less than $1/10^8$ per vector genome per generation." Also, the vector may carry no resistance to antibiotics used clinically or in agriculture. The provision that antibiotic resistance markers of medical or agricultural importance are not to be used in the vector should prevent any inadvertent advantage for recombinant DNA-bearing vectors that encounter antibiotics in the environment.

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[1] The history of the U.S.-EMBO Workshop and the April 6-07, 1978, working group is discussed in detail in Pt. III of this document under the heading "Recombinant DNA Experiments Involving Viral DNA" and the report of the working group appears as App. E to the accompanying environmental impact assessment.

[3] Under the proposed revisions, HV1s other than *E. coli* K-12 need not offer a distinct advantage over *E. coli* K-12 host-vectors, need not be capable of modification to HV2 and HV3, and need not be class I etiologic agents.

Certification of host-vector systems. A new section has been added detailing the responsibility for certification of HV1, HV2, and HV3 systems, the types of data to be submitted, and the mechanisms for distributing strains once certified. The section delineates procedures used by the RAC for the past 2 years and therefore represents no change from practices under the 1976 guidelines.

Review of comments and NIH-proposed guidelines

I have reviewed the biological containment section of the PRG-RAC in the light of comments and suggestions made by participants of the Director's Advisory Committee (DAC) as well as written comments received before and afterward, and have adopted the recommendations of the PRG-RAC with some revisions. An analysis of the specific issues raised by commentators and the basis for my decision follow.

Development of Alternative Host-Vector Systems. Many commentators from the scientific community believe that the PRG-RAC discriminates against alternate host-vector systems other than *E. coli* K-12. They urge development of other systems, maintaining that new systems will be needed increasingly, both in pure research and in industry, and should be certified as soon as possible. It is unlikely, according to one commentator, that agriculture will best be served through the use of *E. coli* K-12 (or *B. subtilis*), and that alternate host-vector systems are therefore essential if the potential of recombinant DNA technology for agriculture is to be realized. In view of the support evident at the 1976 DAC meeting for NIH to encourage development of alternate host-vector systems, one commentator expressed disappointment that there was not now a large NIH contract program in this area.

Others view the introduction of alternate HV systems with some misgivings. It was pointed out, for example, that if uncertainty continues to surround research with so well-studied an organism as *E. coli* K-12, our ignorance must be that much greater with regard to any other organism—its ecological involvement, the organisms with which it can exchange DNA, etc. Moreover, the guidelines, which have been developed around the use of *E. coli* K-12, are primarily focused on dangers to man, and the introduction of new systems may affect other life forms with which we should be equally concerned. In the view of commentators who urge restraint, the larger the number of systems certified, the greater the problem of monitoring the work.

Clearly, however, research addressed to the development of other host-vector systems must proceed. This is particularly evident in the agricultural sector, where the potential for immediate benefits to man is great. At present, a number of alternate systems, including those using *B. subtilis* and *Saccharomyces cerevisiae*, are being developed by NIH grantees. The interest shown by numerous investigators in developing new host-vector systems means that NIH need not develop a special program to promote research in this area.

I appreciate and understand the concern of those who urge deliberate caution. I would stress that the same considerations of safety and risk associated with the use of *E. coli* K-12 will also apply to any new host-vector systems to be certified in the future.

Risk Assessment. Many commentators advocate more studies in risk assessment. It has been maintained that assumptions about biological containment may not be valid and that all components should be tested. Concern has been expressed that the biological containment safety systems may fail altogether.

Some risk assessment studies are prohibited by the 1976 guidelines. Under the PRG-RAC, however, the Director, NIH, on recommendation of the RAC, would have discretion to permit such risk assessment experiments by granting a waiver from a specific prohibition. There was virtually unanimous support for this discretion at the DAC hearing in December 1977. Of course, its exercise must be consistent with standards of due process for the scientific community and the public.

Risk assessment studies are proceeding both within and outside the United States. For example, the "polyoma" experiment,[4] which was delayed in this country because of litigation and the renovations necessary to meet the extremely stringent P4 requirements, has now begun here, and a similar experiment is proceeding in Europe. The work of Robin Holliday in assessing statistical probabilities of biological accidents is also noteworthy (see appendix P of the October 1977 Environmental Impact Statement).

NIH is committed to the conduct and support of risk analysis studies to determine the extent to which certain potentially harmful effects from recombinant DNA molecules may occur. It is intended that the NIH P4 facilities both in Bethesda, Md., and at the Frederick Cancer Research Center will serve as a focal point for many such studies. Provision has already been made to share these facilities with non-governmental scientists.

It should be stressed that prior to certification as EK2, each candidate EK2 host-vector system is analyzed in great detail by the RAC and NIH. Much data must be submitted, a good deal of which is risk assessment data.

Safety of E. Coli K-12. In 1976, there was considerable comment regarding the use of *E. coli* K-12 as a host, including recommendations that its use be prohibited. Some recent commentators have also questioned the safety of *E. coli* K-12, noting that the Falmouth Workshop proceedings had not been published for public review. On the other hand, one commentator urged that, base on the safety of *E. coli* K-12, essentially all experiments employing *E. coli* K-12, be exempted from the Guidelines. An extensive discussion of *E. coli* K-12 together with new scientific information on its safety are presented in part III of this document and in a special section of the Environmental Impact Assessment.

The proceedings of the Falmouth Workshop have now been published in the May 1978 issue of Journal of Infectious Diseases. Reprints are available from the Office of Recombinant DNA Activities, NIH, Bethesda, Md. 20014. As noted in a letter of July 14, 1977, from Dr. Sherwood Gorbach, moderator of the Falmouth Workshop and Chief of Infectious Disease and Professor of Medicine at Tufts University School of Medicine, "The participants arrived at unanimous agreement that *E. coli* K-12 cannot be converted into an epidemic pathogen by laboratory manipulations with DNA inserts."

Comments on Specific Containment Levels. One commentator sought clarification of section II-D-1-a of the PRG-RAC, which defines HV1. According to the second sentence, "The host should have a low potential for survival in its natural environment." As the commentator noted, " 'natural environment' could be ambiguous, in practice. Presumably many of the host cells that people may wish to use have no natural environment other than the laboratory." I referred this comment to the RAC at its April 27-28, 1978, meeting. The RAC agreed that this sentence is ambiguous and recommended that it be deleted. I have done so in the PRG-NIH.

A question was raised on whether HV1 hosts could be wild type organisms or if they are always "meant to harbor containment mutations." If wild type organisms can qualify as HV1, then the definition of HV1 should be reworded to state this explicitly. The answer to the question is that if wild type organisms meet the criteria for HV1, they may be certified

[4] Two NIH virologists, Drs. Wallace Rowe and Malcolm Martin, are linking viral DNA from the mouse polyoma virus with the DNA of bacterial plasmids and bacteriophages and inserting this recombined DNA into a weakened strain of *E. coli*. The bacteria will then be injected into or fed to mice to determine the effects, if any, of the viral DNA.

as HV1. However, I see no need to modify the definition to state this explicitly.

One commentator thought the standards for HV1 should be significantly relaxed and that NIH approval should not be necessary. He proposed that the Guidelines state that "wild type isolets of any bacterial species not known to be pathogenic to humans, to domestic animals, or to agriculturally important plants may be used as an HV1 host-vector system, provided that all components of recombinant DNA molecules introduced into such a host-vector system, are derived from other prokaryotic organisms within Etiologic Agent Class 1." I have rejected this suggestion since I believe it prudent, at least for the present, to have higher standards and to require NIH approval before a system may be called HV1. Some commentators have urged that the requirement for independent confirmation of relevant phenotypic and genotypic traits before certification at the HV3 level should also be applied at the HV2 level. There are two objects of such testing: (1) To determine whether a system already approved has changed its characteristics before a new sample of it is distributed (for example, whether the amber mutations for phage systems are still present), and (2) to repeat independently all the safety tests required before each new system would be certified. The first could be done easily and is sufficient to confirm the safety characteristics; the second is cumbersome and difficult. It should be pointed out that the RAC and its working groups that review the data on proposed HV2 systems are, in effect, conducting an independent check and know this area of research well. Further, the Committee may request that additional experimental data be submitted as part of its review. NIH believes these controls to be sufficient. Consequently, the requirement for an independent check at the HV2 level is deemed unnecessary.

For the HV3 level of containment, some objections have been raised to the requirement banning antibiotic-resistance markers. Antibiotic resistance can serve as a valuable marker in experiments with organisms bearing recombinant DNA. The ban at the HV3 level, however, is prudent inasmuch as organisms rendered antibiotic resistant would be less amenable to control should they escape from the laboratory. This requirement also allows only a certain class of certified HV2 systems to qualify for HV3. Therefore, attempts to develop systems that meet these HV3 criteria should simultaneously upgrade the HV2 systems in use, since it is to the experimenter's advantage to use those HV2 systems with the greatest likelihood of meeting HV3 criteria.

Certification. A number of commentators have urged more precise criteria for biological containment systems. They feel that criteria should be as objective as possible and should be framed in terms of performance, as in the case of physical containment (for example, safety cabinets). It should be stressed that specific objective criteria do exist for EK2 host-vector systems. These, however, do not appear in the Guidelines themselves, but rather as information in the Environmental Impact Statement, Appendix H, entitled "Certification of EK2 Host-Vector Systems." To insure that detailed material on certification of host-vector systems is readily accessible, NIH will publish specific criteria in a standarized format in the *Recombinant DNA Technical Bulletin.* Specific instructions concerning the type of data to be submitted to NIH for proposed EK2 systems involving either plasmids or bacteriophage lambda in *E. coli* K-12 are available from the NIH Office of Recombinant DNA Activities, and a statement to this effect is included in the PRG-NIH.

Many problems persist for setting general criteria that could be applied to all organisms for possible certification as HV2 and HV3. For example, with *B. subtilis,* which forms spores, safety would depend on nonsporulating derivatives. Some commentators urged that all new systems be certified with deliberate caution, and that criteria and evidence should be a matter of public record before decisions are made. The *B. subtilis* system was cited as a case in point; extensive public analysis and debate should precede certification.

I agree that prior notification to the public in the FEDERAL REGISTER should be given when the RAC considers applications for certification. (It should be noted that all meetings of the RAC are announced in the FEDERAL REGISTER.) I also agree with the suggestion that the RAC should have a more fixed schedule of meetings throughout the year so that the public and scientific communities may know the schedule of events clearly.

The entire section (II-D-2-a) on responsibility for certification of host-vector systems has been rewritten in the PRG-NIH to clarify this process.

Distribution of Certified Host-Vectors. Some commentators have suggested that NIH distribute HV1 systems as well as HV2 and HV3 systems. Language has been placed in the PRG-NIH indicating that, where appropriate, HV1 systems other than *E. coli* K-12 may be sent by NIH to investigators.

Concern has been expressed about culture contamination and how this problem would be addressed. The PRG-NIH provides that if NIH propagates any of the host strains or phages, it will not distribute the culture before sending a sample to the investigator who developed the system or to an appropriate contractor for verification that "the material is free from contamination and unchanged in phenotypic properties." The PRG-NIH also assigns to the investigator the responsibility for "insuring the integrity of physical containment (e.g., biological safety cabinets) and biological containment (e.g., genotypic and phenotypic characteristics, purity, etc.)."

Distribution of certified host-vector systems has raised comment relating to the protection of proprietary information and patent rights, for this section of the Guidelines seems to mandate distribution and might conflict with patent protection. NIH has carefully considered such protection. Language has been included in the PRG-NIH (in section IV-C) allowing RAC review for certification at the request of the private sector. The language notes, however, that interested individuals should consider filing for patent protection before submitting information to DHEW. To be consistent with the institutional patent agreement policies of the Department of Health, Education, and Welfare, support is accorded the concept of protection of proprietary and patent rights within the bounds of due process for public review.

III. CONTAINMENT GUIDELINES FOR COVERED EXPERIMENTS

REVIEW OF RAC-PROPOSED GUIDELINES

A major concern of all individuals who have participated in establishing guidelines for recombinant DNA research is that any guidelines that are drafted and adopted be reassessed periodically and changes made when warranted by new information and/or experimental data. In keeping with this responsibility, the RAC compiled additional information pertaining to risk assessment in recombinant DNA research. This information is in the following forms:

1. Consultations with scientists with expertise in the areas of evolution, plant biology, bacteriology, virology, and human and animal infectious diseases;

2. Reports from scientific meetings dealing with the potential biohazards of recombinant DNA research (for example, the *Tenth Miles International Symposium on Recombinant Molecules—Impact on Science and Society,* Cambridge, Mass., June 1976; the *National Academy of Sciences Forum on Recombinant DNA Research,* Washington, D.C., March 1977; *Genetic Engineering for Nitrogen Fixation,* Brook-

haven, N.Y., March 1977; and the *Workshop on Studies for Assessment of Potential Risk Associated with Recombinant DNA Experimentation*, Falmouth, Mass., June 1977);

3. Results from experiments specifically designed to test (a) the survivability and colonizing ability of *E. coli* K-12 and EK2 host-vector systems, (b) the transmissibility of plasmids and phage vectors, (c) the potential of *E. coli* K-12 for pathogenicity, and (d) the potential of genetic exchange between diverse bacteria and between eukaryotic and prokaryotic organisms.

Each category of experiments in part III of the original guidelines was then extensively examined, applying the following criteria to the new information:

- The degree to which the DNA segment has been purified away from other genes and shown to be free of harmful characteristics;
- The potential biohazard associated with the DNA of the cell or microorganism that serves as the DNA source (e.g., genes for toxin production);
- The potential biohazard associated with the vector that serves to transmit the source DNA to a recipient host cell;
- The ability of the vector to survive in natural environments or habitats;
- The kinds and number of different organisms that are susceptible to infection by the vector or recipient;
- The potential biohazard of the recipient host cell that serves to replicate the recombinant DNA molecule;
- The ability of the recipient cell to survive in natural environments of habitats;
- The ability of the recipient cell to transmit the recombinant DNA molecule to other cells capable of surviving in natural environments or habitats;
- The potential of the recipient cell to obtain the source DNA by natural means; and
- The evolutionary relatedness of the DNA source to humans. The potential dangers are considered to increase as the organism providing the source DNA approaches humans phylogenetically. Thus, source DNA from primate cells is considered to have greater potential danger than source DNA from prokaryotes.

To present more clearly the changes in containment levels proposed by the PRG-RAC, a table was prepared for use at the December 1977 meeting of the Advisory Committee to the Director, which compared the containment levels in the PRG-RAC with those of the 1976 guidelines. This table has now been expanded with a third column to show the containment levels of the proposed revised guidelines which are now being proposed by NIH (called PRG-NIH). The table appears as appendix A to the accompanying Environmental Impact Assessment.

The remainder of this section summarizes a number of the proposed changes comparing the 1976 guidelines with the PRG-RAC. (Not all the changes are discussed here; certain items in which the PRG-NIH differs significantly from the PRG-RAC are considered below in the section entitled "Review of Comments and NIH Proposed Guidelines.") The numbers in parentheses indicated the line numbers on the table to which the proposed revision applies.

The principal changes reflected in the table are as follows:

- Several categories of experiments (primarily those involving prokaryotes that are exchangers of genetic information with *E. coli* in nature) are no longer subject to the provisions of the PRG-RAC due to the changes in the definition. (See lines 20, 21, 27, 46, and 47.)
- Shotgun experiments involving birds and mammals other than primates were the subject of lowering of containment from P3+EK2 to P2+EK2. This action reflects the increased confidence of the RAC in the EK2 host-vector systems. (See lines 4 and 5.)
- Another category which the RAC decided was in need of revision was that pertaining to the cloning of DNA from organisms producing a toxic product. This was clarified in the PRG-RAC by specifying whether or not polypeptide toxins are produced, and setting containment levels accordingly. Polypeptide toxins are specified, since they might be encoded by a single gene or cluster of genes. Toxins of other chemical structure would not result from a single gene or cluster of genes. (See lines 8, 9, 10, 11, 12, 16, 17, and 19.)
- For several categories of experiments, it is proposed that the investigator have the option of working at P2+EK1 or P1+EK2 rather than the P2+EK1 levels previously specified. This again reflects confidence in the EK2 systems. (See lines 7, 14, and 15.)
- The lowering of containment for experiments with rigorously characterized clones free of harmful genes was revised to provide more fexibility. Under the PRG-RAC, institutional biosafety committees (IBC's) would be able to lower containment by a single level. The IBC should consider the purity, extent of characterization, and harmlessness of the clone before allowing such lowering. Reduction of containment by more than one level would require approval by NIH. Under the 1976 guidelines, NIH had the option of lowering containment down to certain specified levels or not lowering it at all. The PRG-RAC would allow NIH to consider all available data for the clone and to lower containment accordingly.

In addition, the section now applies to rigorously characterized clones from any permissible experiment in *E. coli* K-12. Under the original guidelines, containment for *E. coli* K-12 clones containing characterized and harmless portion of viruses and plasmids could not be lowered.

The rationale for these proposed changes is explained in further detail in the Environmental Impact Assessment.

REVIEW OF COMMENTS AND NIH PROPOSED GUIDELINES (GENERAL)

Rationale

Part III of the guidelines received the most extensive comment of any section during the development of the original guidelines in early 1976. While there was also much discussion of this part in the PRG-RAC, the issues raised did not primarily address the proposed changes in the containment levels but more general topics such as the need for a rationale for each of the changes.

A number of commentators asked that the rationale for the classification of pemissible experiments be clearly spelled out. It was pointed out that (1) the part on permissible experiments is especially difficult for a lay person to understand, (2) the whole categorization is dependent upon investigatorial confidence rather than documented fact, and (3) the quantification of containment levels, the means by which the levels were decided, and the rationale for raising and lowering these levels are not clear.

In general, the classification may appear somewhat arbitrary, because it depends in large part on the scientific judgment of the RAC rather than on demonstrable risk, since there is actually no scientific evidence of hazard in any recombinant DNA experiment.

The rationale for classifying different recombinant DNA experiments at different containment levels was explained in the "Decision of the Director, National Institutes of Health, To Release Guidelines for Research on Recombinant DNA Molecules," which was published along with the current guidelines in the FEDERAL REGISTER on July 7, 1976, as follows:

> The guidelines assign different levels of containment for experiments in which DNA from different sources is to be introduced into an *E. coli* K-12 host-vector system. The variation is based on both facts and assumtions. There are some prokaryotes (bacteria) which constantly exchange DNA with *E. coli*. Here it is assumed that experimental conditions beyond those obtained in careful, routine microbiology laboratories are super-

fluous, because an exchange experiments have undoubtedly been performed already in nature.

In every instance of artificial recombination, consideration must be given to the possibility that foreign DNA may be translated into protein (expressed), and also to the possibility that normally repressed genes of the host may be expressed and thus change, undesirably, the characteristics of the cell. It is assumed that the more similar the DNAs of donor and host, the greater the probability of expression of foreign DNA, or of possible derepression of host genes. In those cases where the donor exchanges DNA with *E. coli* in nature, it is unlikely that recombination experiments will create new genetic combinations. When prokaryote donors not known to exchange DNA with *E. coli* in nature are used, however, there is a greater potential for new genetic combinations to be formed and be expressed. Therefore, it is required that experiments involving prokaryotic DNA from a donor that is not known to exchange DNA with *E. coli* in nature be carried out at a higher level of containment. Recombination using prokaryotic DNA from an organism known to be highly pathogenic is prohibited.

There are only limited data available concerning the expression of DNA from higher forms of life (eukaryotes) in *E. coli* (or any other prokaryote). Therefore, the containment prescriptions for experiments inserting eukaryotic DNA into prokaryotes are based on risks having quite uncertain probabilities.

On the assumption that a prokaryote host might translate eukaryotic DNA, it is further presumed that the product of that foreign gene would be most harmful to man if it were an enzyme, hormone, or other protein that was similar (homologous) to proteins already produced by or active in man. An example is a bacterium that could produce insulin. Such a "rogue" bacterium could be of benefit if contained, a nuisance or possibly dangerous if capable of surviving in nature. This is one reason that the higher the phylogenetic order of the eukaryote, the higher the recommended containment, at least until the efficiency of expression of DNA from higher eukaryotes in prokaryotes can be determined.

There is a second, more concrete reason for scaling containment upward as the eukaryote host becomes similar to man. This is the concern that viruses capable of propagating in human tissue, and possibly causing diseases, can contaminate DNA, replicate in prokaryote hosts and infect the experimentalist. Such risks are greatest when total DNA from donor tissue is used in "shotgun" recombinant experiments; it diminishes to much lower levels when pure cloned DNA is used.

The structure of the classification for permissible experiments is based, therefore, on assumptions governing potential risk. It should be emphasized again that although recombinant DNA experiments have now been performed for over five years in hundreds of laboratories throughout the world with hundreds of thousands of different recombinant DNA molecules produced, no case of hazard has been demonstrated.

Part III of the guidelines assigns to each specified class of experiments a level of physical containment and a level of biological containment at which the experiment shall be performed. As noted before, there is 10,000- to 100,000-fold protection in going from a class I or II biological safety cabinet to a class III biological safety cabinet (i.e., from P3 to P4). Similarly, in going from P1 to P3 there may be a 10,000- to 100,000-fold increase in safety. For biological containment, there is the criterion for HV2 systems that "escape of the recombinant DNA either via survival of the organisms or via transmission of recombinant DNA to other organisms should be less than $1/10^8$ under specified conditions." However, that criterion is not relative to the HV1 host-vector systems but absolute; thus, this might be a characteristic found for some host-vectors in the HV1 system, but it is mandated for all HV2 systems. This level was chosen, it was pointed out, because it represents a practical limit which one can measure experimentally.

Use of E. coli *K-12*

A number of comments were made concerning the use of *E. coli* host-vector systems. It was observed that because *E. coli* K-12 is currently a "poor" pathogen doesn't mean that it might not be converted to a "good" pathogen with the addition of one or two genes; the enfeebled nature of *E. coli* K-12 "is presumably the consequence of mutation(s) introduced during its laboratory passage," but that perhaps different strains of K-12 with different histories may not all be similarly enfeebled.

Further, it was claimed that the failure to convert K-12 to a pathogen by the use of certain plasmids or *Salmonella* genes is not definitive; to be definitive, we must have the detailed nature of the mutations in K-12 "which prevent the expression of pathogenicity." Also, it was noted that there is no way to assess the absolute risk associated with these experiments, and that it is important to assess the potential harm not only to man but to plants, animals, and the enviroment.

Another commentator urged that this section be supplemented with the evidence from the Falmouth Conference to show that the potential risk is minimal. A commentator cited the potential risk on the basis that "virtually any highly conserved physiologically active eukaryotic protein * * * or fragment thereof could be highly toxic when introduced out of context by a bacterium which received the appropriate gene in a recombination experiment." This criticism of the *E. coli* K-12 system does not detract from the scientific knowledge over the past two years of the great safety of this system. This evidence is presented in detail in the Environmental Impact Assessment. I agree that different strains of K-12 with different histories may not all be similarly enfeebled and that failure to convert K-12 to a pathogen to date does not prove it can never happen. However, the safety of *E. coli* K-12 has been clearly shown and there is no need to limit or specify particular strains for EK1. After 30 years of work with many different strains, there is still no known pathogenic *E. coli* K-12 strain. Thus, there is presumptive evidence that all K-12 strains are safe. They are well suited for laboratory experiments because they take up DNA easily, but their cell wall makes them unsuited to compete in nature with wild-type *E. coli*.

On the basis of the Falmouth Conference (which is discussed further in the Environmental Impact Assessment), the conclusion can be drawn that it is essentially impossible for *E. coli* K-12 to be transformed by recombinant DNA into a wild-type, pathogenic *E. coli*. An *E. coli* K-12 containing toxic genes through recombination could theoretically present a risk to a laboratory worker who accidentally ingested it; but it would only be to that laboratory worker. There is evidence to show that harmful genes will have a very low probability of being transferred from *E. coli* to another organism. The plasmids used at the HV2 level are engineered so that they neither self-transfer nor transfer when another plasmid induces conjugation. Thus, the high degree of safety of this system is clear and explains why it is preferable to any other host-vector system at present.

General Classification

Disagreement was expressed over whether the PRG-RAC was too stringent or too lax. Those arguing the former position maintain that the guidelines should be relaxed even further because all the experimental evidence gathered and analyzed in the past 2 years indicates that the initial fears concerning the potential hazards were extremely exaggerated; moreover, the benefits to be derived from the research are great. Also, it is pointed out that recombinant DNA experiments not allowed under the current NIH guidelines are proceeding with the approval of responsible national committees in a number of European countries. Those opposing this view argue that there is a lack of experimental data for a sound evaluation of the potential risks, and the fact that a recombinant DNA experiment is permitted in Europe is irrelevant to the establishment of standards in the United States.

Recombinant DNA Experiments Involving Viral DNA

Many of the commentators agreed that both the original guidelines and the PRG-RAC were overly stringent with regard to virus experiments. In commenting on the PRG-RAC, the EMBO Standing Committee on Recombinant DNA Research wrote:

> The EMBO Committee believes that the containment categorization of experiments with animal virus DNA's which is proposed by the NIH Advisory Committee is too indiscriminate and excessively stringent considering the proposed classification of experiments with other classes of DNA and the longstanding, accepted safety precautions for handling intact virus particles and viral nucleic acids * * *. The EMBO Committee proposes that it would be more reasonable either to consider experiments with viral DNA on a case-by-case basis or to produce a detailed set of recommended categories for experiments with specific viral DNA's. The EMBO Committee hopes in the near future to establish an ad hoc international group of virologists to draw up such proposals.

In response to this suggestion (i.e., for an international group of virologists to consider this issue), a joint U.S.-EMBO Workshop To Assess Risks for Recombinant DNA Experiments Involving the Genomes of Animal, Plant, and Insect Viruses was held in Ascot, England, on January 26-28, 1978. The workshop was attended by 27 distinguished scientists from the United States, the United Kingdom, West Germany, Finland, France, Sweden, and Switzerland. The report of the "Ascot" Workshop was published in the FEDERAL REGISTER on March 31, 1978, and appears as appendix E to the accompanying Environmental Impact Assessment. The workshop concluded:

> The probability that K-12 organisms carrying viral DNA inserts could represent a significant hazard to the community was so small as to be of no practical consequence * * * viral genomes or fragments thereof, cloned in *E. coli* K-12 using approved plasmid or phage vectors, pose no more risk than work with the infectious virus or its nucleic acid and in most, if not all cases, clearly present less risk. In fact, the workshop participants agreed that cloning of viral DNA in *E. coli* K-12 may provide a unique opportunity to study with greatly reduced risks the biology of extremely pathogenic and virulent viruses.

On April 6-7, 1978 (as announced on March 17 in the FEDERAL REGISTER), a working group sponsored by the RAC, composed of distinguished American microbiologists, met to review the report of the "Ascot" Workshop. The report of this working group appears as appendix F to the accompanying Environmental Impact Assessment. The working group unanimously endorsed the "Ascot" report with certain minor amendments. Their report included recommended new language to be inserted in the PRG-NIH in place of the sections dealing with viruses in the PRG-RAC. This report was presented to the RAC at its April 27-28, 1978, meeting, and was unanimously endorsed by the RAC with certain minor amendments. I have accepted these recommendations of the RAC, with certain additional minor amendments, and these now constitute the sections dealing with viruses in part III of the PRG-NIH.

Recombinant DNA Experiments Involving DNA from Plants and Plant Pathogens

One of the comments made at the December 1977 meeting of the Advisory Committee to the Director, NIH was that "the NIH guidelines do not adequately deal with the use of recombinant DNA in plants * * *" Other commentators have expressed similar sentiments, and the suggestion has been made that "a subcommittee be formed to deal with plants and plant pathogens and make specific recommendations for revision of the guidelines." In response, a Workshop on Risk Assessment of Agricultural Pathogens, composed of distinguished American plant pathologists, was held on March 20-21, 1978 (as announced on March 6 in the FEDERAL REGISTER). Sponsored by the U.S. Department of Agriculture, the National Science Foundation, and the NIH, the report of this workshop appears as appendix G to the accompanying Environmental Impact Assessment. The report was presented to the RAC at its April 27-28, 1978, meeting and was unanimously endorsed by the RAC with certain minor amendments. I have accepted these recommendations of the RAC with certain additional minor amendments; these involve changes in the PRG-NIH in sections dealing with the use of plants and plant pathogens in recombinant DNA research.

Using the 10 criteria previously discussed in light of what is known today, I believe the revisions in containment standards proposed by the PRG-NIH are sound. The changes in containment standards in the PRG-NIH are discussed below in greater detail for each of the subsections of part III.

SPECIFIC CONSIDERATIONS

Section III—Opening Paragraphs

As discussed above in part I of this document, the section of the guidelines numbered III-A in both the 1976 guidelines and the PRG-RAC and entitled "Experiments That Are Not To Be Performed" has been moved in the PRG-NIH to become section I-D entitled "Prohibitions." This leads to a renumbering of the remaining subsections of part III of the PRG-NIH as compared to the PRC-RAC.

Two new paragraphs have been inserted at the beginning of part III of the PRG-NIH. The first reminds the reader to consult part I "where listings are given of prohibited and exempt experiments."

The second inserted paragraph is a "general flexibility clause." Insertion of such a "clause" was recommended by the RAC at its April 27-28, 1978, meeting. It recognizes that the classification of experiments given in part III will necessarily be imperfect, as investigators in the future devise new ways to conduct recombinant DNA experiments not currently foreseen and therefore not explicitly considered in the guidelines. Also, new data may become available showing that certain particular experiments currently assigned a particular containment level are, indeed, clearly more (or less) safe than envisioned at this time. Therefore, this "clause" states that "changes in these levels for specific experiments (or the assignment of levels to experiments not explicitly considered here) may be expressly approved by the Director, NIH, on the recommendation of the Recombinant DNA Advisory Committee (RAC)."

Section III-A-1-a. Shotgun Experiments into E. coli *K-12 with Inserted Eukaryotic DNA*

At a number of places in this subsection the principal investigator is allowed to choose between two combinations of containment procedures. For example, in several instances one is permitted to use P2+EK1 or P1+EK2. This was endorsed by some commentators but questioned by others. This concept of flexibility was addressed in part II of this document. I also wish to point out that the concept is not a new one—it was allowed under the original guidelines. Based upon events of the past 2 years, the RAC merely proposed that this principle be extended to certain specified additional cases where they believe it appropriate. I agree with their proposals and have therefore included in the PRG-NIH all such specific cases of flexibility recommended in the PRG-RAC.

On the other hand, in certain other specific cases (e.g., DNA from birds) the PRG-RAC recommended the containment level be P2+EK2, without the option of P3+EK1. Certain commentators urged that in all cases where the containment level of P2+EK2 is given, the option of P3+EK1 be allowed. However, the RAC felt that in view of their increased confidence in the biological containment offered by the EK2 system, P2+EK2 offers more containment than P3+EK1, and that P2+EK2 without the option of P3+EK1 should be the containment level for certain specified classes of experiments. I accept the view of the RAC and have therefore specified in the PRG-NIH the containment levels of P2+EK2 without the option of

P3+EK1 in every case where it appeared in the PRG-RAC.

The section of this document on "Recombinant DNA Experiments Involving Viral DNA" discussed the "Ascot" workshop report, and the April 6-7, 1978, working group report which endorsed the "Ascot" report. The RAC at its April 27-28, 1978, meeting unanimously endorsed the working group report recommending lower containment levels for deliberate cloning of viral DNA into *E. coli* K-12 (see below for discussion of section III-A-2). One of the reasons given originally for the higher containment level for shotgun experiments involving primate DNA into *E. coli* K-12 was the possible *inadvertent* cloning of viral DNA. In view of their recommendation of lower containment for *deliberate* cloning of viral DNA into *E. coli* K-12, the RAC on April 27-28, 1978, reconsidered primate shotgun levels, and voted unanimously for new language as follows: "*Primates.* P2 physical containment + an EK2 host-vector. Any lowering of containment below these levels (i.e., for purified DNA or characterized clones) cannot be made solely by an institutional biosafety committee but requires NIH approval." I have accepted this new language and inserted it in the PRG-NIH, as well as a similar lowering of containment for shotgun cloning of cold-blooded vertebrate DNA into *E. coli* K-12.

One commentator noted that section III-B-1-a-(1)-(g) of the PRG-RAC entitled "Cloning of Viral Genomes From Eukaryotic Cell DNA" * * * "focuses on cloning integrated retrovirus nucleotide sequences from mammalian cell DNA but says nothing about nucleotide sequences of integrated DNA viruses." This entire section has been eliminated from the PRG-NIH and instead a new subsection III-A-2-a-(3) entitled "Intracellular Viral DNA" has been added to the PRG-NIH which covers both integrated retroviruses and DNA virus sequences; it says, "Physical and biological contaminant specified for shotgun experiments with eukaryotic cellular DNA (See section III-A-1a) shall be used for DNA recombinants produced with integrated viral DNA or viral genomes present in infected cells."

Section III-A-1-b. Shotgun Experiments Into E. Coli *K-12 With Inserted Prokaryotic DNA*

In the 1976 guidelines, the section (III-B-2-(a)-(ii)) dealing with shotgun experiments into *E. coli* K-12 with inserted prokaryotic DNA was subdivided into two sections, i.e., "Prokaryotes That Exchange Genetic Information With *E. coli*" and "Prokaryotes That Do Not Exchange Genetic Information With *E. coli.*" In the PRG-RAC it was assumed that all prokaryotes that exchange genetic information with *E. coli* would be exempt from the guidelines by appearing on the "list of nonnovel exchangers." Therefore, in the PRG RAC the section (III B-1-a (2)) dealing with shotgun experiments into *E. coli* K 12 with inserted prokaryotic DNA actually considered only prokaryotes that did not exchange genetic information with *E. coli.* The problem with this approach was discussed by commentators, focusing especially on the case of *Agrobacterium tumefaciens.* It meant that a prokaryote which exchanges genetic information with *E. coli*, and was therefore properly assigned a low containment level under the 1976 Guidelines, would under the PRG RAC either appear on the "list" and therefore be exempt from the guidelines, or if for some reason it did not appear on the list, the containment level would actually in some cases be raised. This was not the intent of the RAC. Therefore, I proposed to the RAC, and they accepted at their April 27-28, 1978, meeting, that language be reinserted in the the PRG-NIH covering prokaryotes that exchange genetic information with *E. coli* but which do not appear on the list. This section in the PRG-NIH (III-A-1-b-(1)) reads:

Prokaryotes That Exchange Genetic Information [35] *with* E. coli. It is expected that many of the prokaryotes that exchange genetic information with *E. coli* by known physiological processes will be exempted from these guidelines by appearing on the "list of exchangers" (see sec. I E 4).

For those not on the list, the containment levels are P1 physical containment + an EK1 host-vector. In fact, experiments in this category can be performed with *E. coli* K-12 vectors exhibiting a lesser containment (e.g., conjugative plasmids) than EK1 vectors. However, for prokaryotes that are classified [1] as Class 2 the containment levels are P2+EK1.

For prokaryotes that do not exchange genetic information with *E. coli*, the PRG-RAC proposed that P1+EK2 or P2+EK1 conditions apply only in cases of extensive characterization and RAC approval. "Experiments with DNA's from bacteria that are not extensively characterized require P2 physical containment + an EK2 host-vector or P3+EK1. Experiments with DNA's from pathogenic species (class 2 and plant pathogens, see App. B) must use P3+EK2." A number of commentators objected to two different aspects of this subsection of the PRG-RAC: (1) Many felt that experiments involving nonpathogenic prokaryotes should be conducted at P1+EK2 or P2+EK1 without extensive characterization or RAC approval; (2) It was argued that plant pathogens should not be included with CDC class 2 agents as requiring P3+EK2 containment. Both of these comments were referred to the RAC at their April 27-28, 1978, meeting and they agreed with the commentators. Therefore, this Section of the PRG-NIH (III-A-1-b-(2)) reads:

(2) *Prokaryotes that Do Not Exchange Genetic Information with E. coli.* P2 physical containment + an EK1 host-vector, or P1+EK2, except for DNA from class 2 agents, [1] which require P3+EK2.

The EMBO Standing Advisory Committee on Recombinant DNA Research recommends that the containment level for all novel non pathogenic prokaryotic DNA into *E. coli* K-12 be P1+EK1. It is my opinion that it is prudent to retain the levels of P2+EK1 or P1+EK2 for nonpathogenic prokaryotes that do not exchange gentic information with *E. coli.*

The PRG-RAC received substantial criticisms for identifying all agents classified as class 2 in the CDC's publication "Classification of Etiologic Agents on the Basis of Hazard" (Fourth edition, July 1974) as being pathogenic for the purpose of assigning containment levels. Many commentators stated that many of the organisms so classified were harmless and others were of such low pathogenicity that severe safety precautions were unwarranted. It was also pointed out that the pathogenicity of an intact micro-organism and the conjectural hazard of a piece of DNA from such an organism within *E. coli* K-12 were quite different matters. It should be noted that the difficulties in application of the CDC classification for the purposes of these guidelines was recognized in the original guidelines. For example, all species of *Salmonella* are classified as class 2 organisms by CDC. The original guidelines, however, distinguish between the pathogenicity of *S. typhimurium* and *S. typhi* for the assignment of containment levels. I have therefore accepted the suggestion of these commentators and have added footnote 1 to the PRG-NIH. This gives NIH the authority, upon the recommendation of the RAC, to designate certain agents which are listed as class 2 by CDC as class 1 agents for the purpose of these guidelines.

Section III-A-2-a. DNA from viruses of eukaryotes into E. coli K-12

Discussed earlier within part III of this document under the heading "Recombinant DNA Experiments Involving Viral DNA" was the history of the "Ascot" workshop report (App. E to the accompanying environmental impact assessment) and the report of the working group which met on April 6-7 1978 (App. F to the accompanying environmental impact assessment). Section III-A-2 of the PRG-NIH adopts the recommendations of the working group with minor modifica-

tion. It is based on a reassessment made by these experts in the field of virology of the potential hazards of inserting pieces of viral DNA into *E. coli*. I believe the argument presented in the "Ascot" report and the working group report are well founded, specifically that "the probability that K-12 organisms carrying viral DNA inserts could represent a significant hazard to the community was so small as to be of no practical consequence * * * viral genomes or fragments thereof, cloned in *E. coli* K-12 using approved plasmid or phage vectors pose no more risk than work with the infectious virus or its nucleic acid and in most, if not all, cases clearly present less risk." Accordingly, section III-A-2-a of the PRG-NIH has been completely rewritten.

Section III-A-2-b. Eukaryotic Organelle DNA into E. coli K-12

To be consistent with the one step lowering of physical containment described earlier for shotgun experiments with primate DNA, the levels for mitochondrial DNA from primates has been similarly lowered by one step in physical containment in the PRG-NIH as compared to the PRG-RAC.

Section III-A-3. Lowering of containment for characterized or purified DNA preparations and clones

Concern was expressed by several commentators regarding the revisions in the PRG-RAC which would allow the local IBC (with notification to be sent to NIH) to reduce either the biological or physical containment level by one step if (1) the DNA is 99-percent purified and shown to be free of harmful genes prior to its insertion into a recombinant molecule, or (2) if subsequent to insertion the clone is rigorously characterized and shown to be free of harmful genes. In the original guidelines lowering in case (2) could only be done with NIH prior approval.

There was support from several commentators for the changes in this subsection. The rationale is explained in new language inserted into this section of the PRG-RAC, which is retained in the PRG-NIH; i.e.:

> Many of the risks which might conceivably arise from some types of recombinant DNA experiments, particularly shotgun experiments, would result from the inadvertent cloning of a harmful sequence. Therefore, in cases where the risk or inadvertently cloning the 'wrong' DNA is reduced by prior enrichment for the desire piece, or in which a clone, made from a random assortment of DNAs, has been purified and the absence of harmful sequences established, the containment conditions for further work may be reduced.

Some commentators noted the ambiguity and difficulty attendant in the phrase "free of harmful genes." The EMBO Standing Advisory Committee on Recombinant DNA Research reports that "several national guidelines for recombinant DNA research state that containment measures may be relaxed once a cloned DNA fragment has been biochemically characterized and shown to be free of harmful genes (NIH guidelines) or devoid of any known pathogenic characteristic (French guidelines). The EMBO committee believes the latter to be a more feasible requirement, but neither can readily be met, and the committee finds it difficult to suggest what sorts of experimental tests might be devised to meet these requirements."

I agree that "the terms 'characterized' and 'free of harmful genes' are unavoidably vague." However, footnote 3 of the PRG-NIH goes on to list five types of data which should be considered in making this determination.

Some commentators were also concerned that this grant of additional authority to the local IBC's for single step lowering in containment levels might introduce variability in the application of the guidelines. I have considered this possiblity and have decided that the principle of promoting local involvement in the implementation of the guidelines outweighs the difficulties which may be eoncountered in this process. In an attempt to minimize these problems, I have (1) attempted to make all parts of the guidelines as clear, specific, and unambiguous as possible, and (2) expanded the "Roles and Responsibilities" secton to outline functions and responsibilities in greater detail. Also, the guidelines require that the Office of Recombinant DNA Activities at the NIH be notified in writing of such an action. A mechanism is therefore in place to ensure that such actions proceed with an acceptable degree of uniformity.

The question was raised whether a clone, the containment level of which was lowered by the IBC at Institution X, may after shipment to Institution Y still be used at the lower level without review by the IBC at Institution Y. It clearly has been, and remains, the intention of both the RAC and myself that the IBC at the receiving institution must approve the reducton in containment for the handling of the clone in such a situation. The investigator at the receiving institution must handle the clone at the higher level until such permission is granted.

One commentator urged that prior cloning be accepted as a technique for the purification of DNA molecules prior to their reinsertion in a new recombinant DNA molecule. The PRG-RAC specified that purification must be achieved "by physical or chemical techniques." The criterion for the single step reduction in containment levels in this situation is that the DNA preparation be 99 percent pure; I see no reason to so restrict the means by which such purification is attained. I have accepted this suggestion as a means of better serving the needs of the investigator without reducing the margin of safety to the public and the environment, and therefore have stricken from the PRG-NIH the words "by physical and chemical techniques" following the worked "purified."

One commentator noted that the PRG-NIH might be nterpreted as allowing a single step reduction in containment levels for purification of the DNA prior to its insertion into a recombinant DNA molecule, and then a subsequent further single step reduction in containment level once the same molecule was cloned. This was not intended. Therefore, clarifying language has been added in the PRG-NIH stating that an IBC "may give approval for a single step reduction in physical or biological containment on receipt of evidence of characterization of a clone derived from a shotgun experiment and its * * *."

Finally, as noted above in this document under "Section III-A1-a—Shotgun Experiments into *E. coli* K-12 With Inserted Eukaryotic DNA," the RAC recommended at its April 27-28, 1978, meeting (and I have accepted the recommendation and inserted it in the PRG-NIH), that the containment levels for shotgun of primate DNA into *E. coli* K-12 be lowered to P2+EK2. However, on the recommendation of the RAC, a stipulation added in section III-A-1-a of the PRG-NIH is that for primate shotgun "any lowering of containment below these levels (i.e., for purified DNA or characterized clones) cannot be made solely by an institutional biosafety committee but requires NIH approval." Language stating this limitation in authority of the IBC with regard to primate DNA has been inserted into subsection III-A-3 of the PRG-NIH, as has language indicating that any lowering of containment under this section to levels below P1+EK1 requires prior NIH approval.

Section III-B. Experiments with Other Prokaryotic Host-Vectors

Some commentators felt that the PRG-RAC unnecessarily emphasized the use of *E. coli* K-12 and would not allow important recombinant DNA experiments to be done in other prokaryotic hosts. Section III-B describes the use of prokaryotic host-vector systems other than *E. coli* K-12 which have been approved as HVI hosts. It should be remembered that "self-cloning experiments with prokaryotic hosts are exempt from the guidelines under exemptions I-E-2 and I-E-3 and that other experiments involving DNA segments from species that exchange

DNA by known physiological processes are exempt from the Guidelines under exemption I-E-4.

The RAC at its April 27-28, 1978, meeting pointed out that there are certain scientifically important experiments which are very safe but which neither fit the criteria to be exempt from the guidelines, nor the criteria for HVI certification. A new section III-B-2 has been added to the PRG-NIH to cover these cases and assign appropriate containment levels. In these experiments DNA from a prokaryotic host (Host X) is cloned into *E. coli* K-12 (this situation is already covered in sec. III-A-1-b(2) of the guidelines); in the second part of the experiment the recombinant DNA (consisting of DNA sequences from Host X linked to an *E. coli* plasmid or bacteriophage) is returned to Host X and propagated there.

Section III-C. Experiments with eukaryotic host-vectors

A number of commentators felt that the stringent containment conditions required both in the original guidelines and in the PRG-RAC for introduction of recombinant DNA into tissue culture cells, using viruses as vectors, were unwarranted. The EMBO Standing Advisory Committee on Recombinant DNA Research wrote:

> In experiments involving the introduction of foreign DNA into cultured cells of animals using DNA viruses as vectors, biological containment is assured by the very restricted permissive conditions for the host cells; the only routes by which the recombinant molecule might escape are by chance infection of a contaminating microorganism or within a viral capsid and the size of the recombinant molecule may well preclude its encapsidation * * *. For example, cloning of mouse DNA using polyoma virus as a vector and mouse cells as host should not require precautions more stringent than those routinely used for many years in laboratories studying polyoma virus infection of mouse cells and mice. The EMBO Committee finds the proposals for this class of experiments in the revised NIH Guidelines not sufficiently discriminating because they would impose unnecessarily high levels of physical containment for experiments with many eukaryotic DNA's.

Discussed earlier within Part III of this document under the heading "Recombinant DNA Experiments Involving Viral DNA" was the history of the "Ascot" workshop report (See App. E to the accompanying environmental impact assessment, and the report of the working group which met on April 6-7, 1978 (App. F to the accompanying environmental impact assessment). I have accepted the recommendations of the work group and incorporated their suggested revision of this section which now becomes section III-C of the PRG-NIH. The result of this change is that section III-B-3 of the PRG-RAC "Experiments with Eukaryotic Host-Vectors," subparts (a) "Vertebrate Host-Vector Systems," and (b) "Invertebrate Host-Vector Systems," are eliminated; substituted for it in the PRG-NIH is new language derived from the working group report which become section III-C "Experiments With Eukaryotic Host-Vectors," subparts (1) Vertebrate Host-Vector System"; (2) "Invertebrate Host-Vector Systems in which Insect Viruses Are Used To Propagate Other DNA Segments", and (3) "Plant Viral Host-Vector Systems."

Section III-C-4. Plant Host-Vector Systems Other Than Viruses

Discussed earlier within Part III of this document under the heading "Recombinant DNA Experiments Involving DNA From Plants and Plant Pathogens" was the Workshop on Risk Assessment of Agricultural Pathogens, held on March 20-21, 1978, sponsored by USDA, NSF, and NIH. Based on the Workshop report (See Appendix G to the accompaying Environmental Impact Assessment), section III-D of the PRG—NIH has been rewritten.

Section III-C-5. Fungal or Similar Lower Eukaryotic Host-Vector Systems

Both the 1976 Guidelines and the PRG-RAC used the same short paragraph for this section, giving little detail, because they noted "the development of these host-vector is presently in the speculative stage." Since that time a specific host-vector system of this class has been developed, i.e., *Saccharomyces cerevisiae* (baker's yeast), and other similar systems may also soon be proposed. Accordingly, this section (III-C-5) of the PRG-NIH has been expanded to give more specific instructions on appropriate containment levels.

Section III-D. Complementary DNAs

Since specific containment levels for the use of purified cDNA of viral mRNA are now given in section III-A-2-a of the PRG-NIH, a sentence has been added noting this at the beginning of section III-D of the PRG-NIH. Otherwise, the rest of this evoked no comments and remains identical in the PRG-NIH to the PRG-RAC.

Section III-E. Synthetic DNA

Because synthetic DNA is now explicitly included in the PRG-NIH (as discussed in section I of this document), it was necessary to add language to Part III of the PRG-NIH detailing the appropriate containment levels for these experiments. The RAC at its meeting on April 27-28, 1978, approved such language, and it has been inserted in the PRG-NIH as section III—E.

IV. Roles and Responibilities

Review of RAC Proposed Guidelines

This section, as in the 1976 Guidelines, provides an administrative framework for implementation. Modifications to the various roles and responsibilities proposed by the RAC are listed below.

Institutional Responsibilities

Institution. Several changes were proposed in the PRG-RAC as compared to the 1976 Guidelines in the responsibilities of the institution. Responsibilities that were added or further detailed included: (1) a requirement for insuring the training of research personnel and the use of good microbiological technique, and (2) a requirement to determine the need for medical procedures, with recommendations of possible specific practices.

Institutional Biosafety Committees. Membership of the committees was clarified by a recommendation to include other than scientific members. In thePRG-RAC (section III), institutional biosafety committees (IBCs) are given the discretion to approve single-step reductions in containment levels for experiments with characterized clones and purified DNA. The IBC's would be required to notify the NIH Office of Recombinant DNA Activities (ORDA) of these approvals.

Biological Safety Officer. Institutions at which P3 and P4 level recombinant DNA work is conducted would be required to have a biological safety officer, whose specific roles and responsibilities are outlined.

Principal Investigator. The role and responsibilities of the principal investigator would remain basically the same except for the important addition of a requirement for training in microbiological techniques. Responsibility for the determination of the practices necessary for medical surveillance would be relocated to the institution.

NIH Responsibilities

Office of the Director. The responsibilities of the Director would remain unchanged. A sentence was added which clarified the Director's authority to implement the Guidelines and to be the final arbiter in the interpretation of the Guidelines.

Recombinant Advisory Committee. There were no changes in the current responsibilities of the RAC; however, there were clarifications of the scope of some duties, for example, the certification process. The language of the 1976 Guidelines caused confusion among some concerning the certification of EK2 (HV2) and EK3 (HV3) host-vector systems. In practice, the certification process involved a two-step procedure: (1) the RAC's recommendation to the Director, NIH, that

a particular host-vestor system be certified; and (2) certification of the system by the Director, NIH. The PRG-RAC clarifies the fact that a two-step procedure is followed. The rationale for the two-step procedure is that it allows the Director, NIH, to solicit the opinions of additional experts prior to making a final decision on certification.

The RAC's authority to recommend exceptions from the proibitions was also clarified. The 1976 version of the Guidelines envisioned the possibility of the RAC's recommending an exception to the 10-liter limit on culture volume for recombinant DNA's known to make harmful products. The proposed revision would extend the possibility of an exception to the five other classes of currently prohibited experiments. The general rationale for this addition is the RAC's inability to foresee all possible future circumstances and the RAC's desire to specify, within the limits of strict safeguards, the possibility of an exception for compelling social or scientific reasons. A more immediate and specific justification for the paragraph on exceptions from the prohibitions is that the risk-assessment studies necessary for a clearer understanding of the potential biohazards of recombinant DNA research may not be able to be carried out without technical violations of the current Guidelines, unless there is a mechanism for approving exceptions.

REVIEW OF COMMENTS AND NIH PROPOSED GUIDEINES

As in the public hearing on the originally proposed Guidelines in 1976, many public commentators urged openness, candor, and public participation in the revision process, emphasizing shared responsibility and accountability from the local to the national level. We have heeded these suggestions. In addition to holding all RAC meetings in the open and holding a public hearing on the PRG-RAC in December 1977, we have published both the PRG-RAC and now the PRG-NIH in the FEDERAL REGISTER for public comment.

It remains clear, as stated in my 1976 decision, that much of the success of the guidelines will depend on the wisdom with which they are implemented. The recommendations of the PRG-RAC have been carefully weighed along with other public and scientific comments received on the "roles and responsibilities" section. In general, I have adopted the RAC proposals with certain additional modifications based on issues raised by the Director's Advisory Committee and other commentators. The issues I have considered, and a discussion of them follows:

Responsibilities of the institution (general)

Again, as in 1976, this section of the guidelines drew considerable comment directed to the roles and responsibilities of the institution and its several constituents. Generally, commentators requested more information and greater clarification of the structure and operation of the IBC, the function of the biological safety officer, and the duties of the institution. Because of the importance of this section with regard to successful implementation of the guidelines, and therefore safe conduct of this research, these suggestions and comments have been carefully considered. NIH acknowledges its special responsibility in assuming leadership in developing and promoting safety programs relevant to recombinant DNA research. Therefore, as in 1976, another committee chaired by Dr. W. Emmett Barkley, Director, Office of Research Safety, NCI, was convened to address concerns raised. As a result, and in response to a number of commentators' requests, appendix D of the original guidelines has been restored and enhanced to give additional advice on safety matters (see "Laboratory Safety Monograph—A Supplement to the NIH Guidelines for Recombinant DNA Research"). The revised guidelines also retain requirements for emergency plans to cover accidents as well as strengthening the requirement for training of all recombinant DNA researchers in safe laboratory procedures.

The intent of this section, as before, is to integrate safety practice into the conduct of recombinant DNA research and to assign responsibilities for this to the principal investigator institution, IBC, and biological safety officer. Therefore, it is important that these responsibilities be stated in an unambiguous manner. For this reason, and in response to many commentators, Part IV has been restructured, to distinguish in greater detail and more clearly align some of these functions. The appendices contain additional complementary information on roles and responsibilities, including information for IBC's and biological safety officers.

In response to several comments, the scope of review of research has been broadened to cover all recombinant DNA research at an institution receiving funds from NIH for recombinant DNA research, whether or not the specific recombinant DNA project is being funded by NIH. While this increases the responsibility of the institution and the IBC's, it is believed that this revision will enhance the overall safety of the conduct of this research. Furthermore, at the suggestion of one commentator, I have decided to change the name of the biohazards committees to biosafety committees to reflect the spirit of the guidelines more closely.

Several generic comments deserve to be highlighted as they represent significantly increased authority to be delegated to the institution. In 1976, the RAC did not accept commentators' suggestions for requiring local committees to make an independent evaluation of the containment levels required by the guidelines for individual research projects. I therefore stated in the 1976 decision that NIH would not require local institutions to have their committees perform this function, although they would not be prohibited from doing so. Commentators have now noted that in order for an IBC to accomplish its mandated responsibilities under the 1976 guidelines, including reviewing and approving recombinant DNA research projects, it has been necessary for the committee implicitly to determine containment conditions. Therefore, in order to better clarify its role, the assessment of appropriate containment levels is now made an explicit responsibility of the IBC.

In addition, institutions through their IBC's will be given increased responsibility for primary oversight of this research as they have now been delegated the authority from NIH to approve or disapprove proposed recombinant DNA projects. NIH through ORDA will conduct a review of institutional actions, upon registration of the projects, to ensure compliance with the NIH guidelines, thereby maintaining a national standard for the research. This action has been in response to several comments calling for increased local responsibility and a more simplified administrative process with regard to gaining approval for this research to proceed. In view of the impossibility of Federal surveillance to enforce these standards externally, I feel it is essential to increase the authority and responsibility of the local institution. It was also requested that IBC's have a role if legislation in this area is adopted, and this concept is endorsed in the bill report of March 24, 1978, on the Recombinant DNA Act, by the House Committee on Interstate and Foreign Commerce, which says, "It is the view of the committee that the appropriate portions of the administrative requirements of section IV of the NIH guidelines are a reasonable model upon which the Secretary could base administrative regulations. In particular, the current practice in the NIH guidelines of delegating to local biohazards committees most of the responsibility for the inspection of facilities and the approval of the specific safety requirements appropriate to each project or activity is an effec-

tive and relatively inexpensive administrative mechanism."

As in the 1976 decision, a number of recommendations were received regarding the membership of IBC's. In 1976, suggestions were made for broadening IBC representation to include members not only from various disciplines related to recombinant DNA molecule technology, biological safety, and engineering but also to include those knowledgeable in applicable laws, regulations, standards of practice, community attitudes, and health and environmental considerations. Consequently, at that time I recommended in my decision that these diverse points of view be included or made available to the committees. The language in the PRG-RAC requires a diversity of membership, but does not mandate noninstitutional members. In response to several requests, and in view of increased responsibility at the local level, I am now going beyond the RAC proposal and adding a provision that "no IBC may consist entirely of persons who are officers, employees, or agents of, or are otherwise associated with the institution, apart from their membership on the IBC." Other specific categories for membership are not mandated although the PRG-NIH now states that "membership should include individuals from disciplines relevant to recombinant DNA technology, biological safety, and engineering"; that it is recommended that "at least one member be a nondoctoral individual from a laboratory technical staff"; and that the IBC "include members knowledgeable about such matters as applicable law, standards of professional conduct and practice, community attitudes, and the environment."

The possibility of conflict between IBC's and local community oversight committees was raised. With noninstitutional membership on IBC's I believe there is no need to have additional community committees.

A number of other recommendations were received from public commentators relating to more specific issues; they are considered below under the appropriate headings.

Responsibilities of the institution (specific)

Institution. A number of points were raised by commentators concerning health monitoring by institutions. NIH was requested to develop a model for institutional medical surveillance for recombinant DNA research workers. The issue of medical monitoring is one of considerable interest to the NIH. This is a general problem not unique to recombinant DNA research. As one commentator noted, instituting a routine health monitoring and reporting program for personnel engaged in areas of research besides recombinant DNA, such as tumor viruses and pathogenic organisms, is important. However, the state-of-the-art is primitive in terms of what can be done to monitor workers' health generally, but particularly in the area of recombinant DNA research where there is no known hazard. At my request, an NIH committee reviewed this area and has made recommendations as to what such a program may include. This recommendation, which calls for monitoring illnesses, collecting serum samples, and keeping a register of agents handled, is responsive to several suggestions received on this issue, and it has, therefore, been adopted in the PRG-NIH. Additionally, appendix D will include more detailed information on medical surveillance.

Grievance procedures for workers under the guidelines were requested but this is not considered necessary as the Occupational Safety and Health Act (OSHA) rules and regulations already provide such a mechanism. In the 1976 decision it was also noted that OSHA standards and procedures apply to most institutions, so it was not considered necessary then, or now, to require in the guidelines that IBC's insure OSHA compliance. Further, the Federal Interagency Committee on Recombinant DNA Research, which I chair, includes representatives from the Occupational Safety and Health Administration (Department of Labor), assuring cooperation at the Federal level.

One commentator spoke to the need for a biosafety control manager which would be similar to the head of a campus environmental health and safety office. While such a program and manager may be desirable, it is felt that this is an institutional administrative matter and should not be addressed in the guidelines.

Institutional Biosafety Committee (IBC). Several commentators requested more detail on IBC duties and this has been accomplished in the supplement to the PRG-NIH entitled, "Laboratory Safety Monograph." For example, information is included here on facility certification, periodic inspections and monitoring, and a model for IBC operation.

There was concern about the establishment of area biosafety committees and possible jurisdictional disputes between them and institutional biosafety committees. This has been further clarified in the definitions in Part I.

It was suggested that biosafety committee meetings be open to the public. The guidelines currently require only that the minutes be available to the public. In view of possible discussion of proprietary and patentable information, IBC meetings cannot always be open. I do urge, however, that local committees, when possible, have open meetings and suggest that all meetings be announced.

The question was raised concerning possible conflict of interest of local committee members. This is an important point, and I have added a provision prohibiting an individual engaged in, or expecting to be engaged in, or having a direct financial interest in, a recombinant DNA project from being involved in the review or approval of that project.

Concern was expressed about the cost of IBC operations, and suggestions were made that the Government underwrite this expense. Because NIH already pays, through indirect costs, the operations of such committees, I have decided that there is no need at this time to separate them out from other indirect costs of the institutions.

Biological Safety Officer. Because increased authority and responsibility have been given the IBC's, it is appropriate that institutions conducting P3 or P4 level research have someone designated to handle biological safety questions generally.

I have accepted the suggestion that the biological safety officer shall be a member of the IBC because his or her responsibilities are so closely allied to the function of the committee.

Another commentator noted that too much emphasis was placed in the PRG-RAC on the regulatory role of the biosafety officer rather than on his or her role as a technical consultant; therefore, language indicating this latter role has been inserted in the PRG-NIH.

In response to questions on the qualifications of biological safety officers, I note that the officer need not be an M.D. Further, it is not necessary that he or she be engaged in research. Since the passage of the Occupational Safety and Health Act, most institutions have established occupational safety and health departments or programs with institutional safety officers. There are no standard certification procedures for such individuals, although their qualifications, in many cases, could be commensurate with those of a biological safety officer. The supplement to the PRG-NIH entitled, "Laboratory Safety Monograph," provides in greater detail the kinds of qualifications that biosafety officers should have. NIH is developing a training course for campus safety officers, including biological safety officers, and requests for information should be directed to Dr. Emmett Barkley, Director, Office of Research Safety, National Cancer Institute, Bethesda, Md. 20014.

Principal Investigator. In response to several commentators, the steps the investigator needs to take in order to have proposed research approved at

the local and national levels have been delineated in Appendix C to the guidelines which contains documentation of NIH administrative procedures for recombinant DNA research projects.

Considerable attention has been given to the issue of training. Several commentators urged that training standards be set by the NIH, preferably in the guidelines. Other commentators wanted the guidelines to direct the institution or IBC to set standards for training; however, some opposed this view. Still others wanted investigator competency evaluated or certified after training had been undertaken. It should be noted that the PRG-RAC represented a strengthening of training requirements, compared to those in the 1976 guidelines. Commentators remain concerned regarding the quality and uniformity of such training. The NIH is responding to this by placing as a high priority the development of training standards and courses. Currently, NIH is supporting a Working Panel of the American Society for Microbiology (ASM) which is considering standards of training in microbiological techniques for recombinant DNA research. When a report is submitted to NIH, it will be shared with institutions, IBC's, and principal investigators for their use. At this time, however, national certification should not be attempted until the ASM/NIH criteria for training have been formulated and subsequently evaluated. It should be noted that, aside from Nuclear Regulatory Commission standards for training for radioisotope work, there are apparently no other formal training criteria presently required for biomedical research. Thus, the work of the ASM Panel will be establishing a precedent. It is for these reasons that I feel NIH should proceed carefully and in stages, at the same time promoting safety training for researchers. Accordingly, NIH will develop courses based on these standards of training and make them widely available.

Responsibilities of the NIH (General)

Due Process Considerations. A focus of public comment at the December hearing was on "procedural due process," to insure public participation in the development of NIH recombinant DNA policies. Much of the public testimony and comment in letters thereafter focused on public representation on committees. Also stressed was the need for public notice of all meetings, and for procedures to insure public participation in the exercise of responsibilities by the RAC, the Office of the Director, NIH, and the Advisory Committee to the Director, NIH.

Several commentators specifically urged that the guidelines spell out the procedures to be used for the following:

- To develop and amend the list of "non-novel experiments";
- To permit the Director, on the advice of the RAC, to grant exceptions from prohibited experiments, such as for risk-assessment experiments;
- To certify host-vector systems;
- To modify guidelines in the future.

There were also suggestions that guidance be given on how to deal with infractions of the guidelines. Specifically, one commentator suggested that procedures outline in detail:

- How charges of non-compliance could be brought;
- How charges of non-compliance would be evaluated;
- What opportunities would be provided for the principal investigator and his institution to defend themselves against charges; and
- What appeals procedures would be available before the termination of funding or the invoking of other penalties.

Because of the key role of the RAC in the development and monitoring on NIH recombinant DNA policies, a number of comments were directed to its composition and functions. Many commentators focused on the RAC's membership, urging that the guidelines define procedures for nomination and selection of members. Suggestions for potential membership on the RAC included more representation for certain scientific disciplines, such as virology and microbiology; greater representation of individuals skilled in occupational and environmental health and safety; and more public representation, including perhaps a "dissenter" from current NIH policies.

A number of comments concerned Committee operations. The RAC was urged to formalize schedules so that all would know when it would meet over the next 2 to 3 years. Further, it was urged that notices and complete agendas be placed in the FEDERAL REGISTER for each meeting; that all documents for Committee consideration be made available to the public; and that the NIH pay for public witnesses to attend meetings of the RAC.

In response to these comments, Part IV of the guidelines has been reorganized extensively. The responsibilities from the local to national level have been stated and defined more clearly. Further, for NIH responsibilities, procedures suggested by commentators have been specified to afford opportunity for public comment. A special appendix to the guidelines includes relevant implementation documents from ORDA that explain the administration of the NIH guidelines at the local and national levels.

From the beginning, the NIH has gone to great lengths to insure procedural due process for the public and scientific communities. The RAC conducts all meetings in the open, and files notice of each meeting in the FEDERAL REGISTER. All the documents listed on the agenda of the RAC meetings have been available to the public. Additionally, the Advisory Committee to the Director, NIH, has provided a public forum on the 1976 guidelines and now on the proposed revisions. The public hearing in February 1976 on the originally proposed guidelines resulted in extensive revision of that proposal. The PRG-RAC was published in the FEDERAL REGISTER on September 27, 1977, for public comment, and the meeting of the Director's Advisory Committee held in December 1977 was announced in the FEDERAL REGISTER. In addition to a general invitation for public testimony, the NIH provided funds for witnesses from the public, private, and scientific sectors to attend and present their views.

The proposed reorganization of Part IV has more clearly defined a structure for responsibilities at the local and national level, with opportunity for public and scientific participation. It makes more formal a process that has been occurring informally. Flexibility, however, remains essential to avoid unnecessary and protracted delays in decisionmaking. Clearly a full panoply of review, including a public hearing, is not essential for most of the functions under the guidelines. For many functions, the need for public review can be met through publication in the FEDERAL REGISTER. For certain responsibilities comment may be solicited. Because procedures by which policies will be developed at the national and local levels are of key importance, notice for major policy initiatives is required. I believe the reorganization of Part IV achieves that goal.

Application to the Private Sector. Several commentators spoke on the application of the NIH guidelines to the private sector. Specifically, the NIH was urged to provide, voluntarily, to the private sector, the following:

- Advice on interpretation of the guidelines;
- Registration of projects;
- Certification of host-vector systems;
- Advice on the operation of institutional biosafety committees; and
- Protection for patent and proprietary information.

Prior to the release of the guidelines in June 1976, representatives of private industry were invited to NIH to be briefed on the guidelines. Since the release of the guidelines, several other meetings with representatives from the private sector have been held. Commerce Department representatives on the Interagency Committee

played a lead role in working with private industry leading to the agreement of relevant industries to abide by the safety standards of the NIH guidelines.

Many of the services provided by the NIH to its grantees and contractors had not been extended to the private sector. After carefully considering the comments at the public hearing and in correspondence received, I now believe the NIH should extend certain services to the private sector in several of the areas suggested by the commentators. A new section has been added to Part IV that provides the opportunity for private industry participation in a voluntary fashion. If legislation is enacted, the NIH Guidelines will serve as the basis for regulation that will encompass the private sector.

Occupational and Environmental Safety. A key concern of all commentators was the need for programs in occupational and environmental safety, that would include health surveillance for laboratory personnel and the community. As I stated in my Decision in 1976, the NIH has a special responsibility for national leadership in programs for laboratory safety. This responsibility is a critical one and we must accept it. Recombinant DNA research policies have stimulated a broad NIH commitment and interest in laboratory safety. The PRG-NIH reflects that commitment. As previously described, there are several training programs that the NIH has undertaken and supported. Several NIH committees are involved in development of policies in this area. The newly updated and expanded supplement to the PRG-NIH entitled, "Laboratory Safety Monograph," reflects the growing experience in this area.

A collaborative effort has been initiated between NIH and the Center for Disease Control (CDC) to establish a mechanism for providing advice, consultation, and if necessary, assistance regarding major accidents in laboratories involved in recombinant DNA research. It was not considered necessary to have a standing "strike force" as suggested by one commentator; however, in the event of an emergency, a team of experts from NIH and CDC could be formed to respond, depending on the nature of the problem.

Several commentators suggested that the NIH examine laboratory work involving genetic techniques other than recombinant DNA research. Indeed, it was recommended that another advisory committee akin to the RAC be established to propose standards for work involving biosafety, generally. I appreciate and understand this concern. The NIH over the past year and a half has created several internal committees that are critically examining different areas where laboratory work is conducted with potential biohazards. These committees are considering possible recommendations for safety standards.

Another commentator also urged the NIH to consider a forum for dealing with social issues related to "genetic engineering." The NIH responsibility to date has been in addressing policy questions involving safety of recombinant DNA research in single cells in the laboratory. I recognize the importance of the potential future application of this and other genetic research to the altering of the genetic character of higher forms of life including man. However, the application of this research to the "genetic engineering" of man is clearly far from imminent. In light of public concern, a study is warranted of the ethical, legal, and social implications of these techniques. The National Commission for the Protection of Human Subjects of Biomedical and Behavioral research considered, but was unable to initiate, a study because of its pressing workload. Such a study, however, should be a key priority for the Commission currently being considered by Congress as part of the legislation to regulate recombinant DNA research.

It has also been suggested that the NIH work closely with other relevant research and regulatory agencies, particularly the Environmental Protection Agency and the Occupational Safety and Health Administration. Indeed, the NIH, from the inception of the Guidelines, has worked to foster cooperation among the Federal agencies. Prior to the release of the Guidelines, representatives from several agencies met at the NIH for a briefing on the Guidelines. After the release of the Guidelines, the question of their extension to the rest of the Federal Government and the private sector prompted the creation of an Interagency Committee. This Federal Interagency Committee on Recombinant DNA Research on which I have served as Chairman, was created by the Secretary of HEW at the request of the President in October 1976. It is composed of all relevant Federal research and regulatory agencies and has provided for coordination of Federal policies concerning recombinant DNA research. In March 1977, the committee developed recommendations for legislation.

It was suggested by a commentator that the NIH address the international implications of control of recombinant DNA research. Indeed, the Federal Interagency Committee issued in November 1977 a thorough and comprehensive review of all guidelines for such research internationally with recommendations for continued cooperation. This report is available from the Office of Recombinant DNA Activities, NIH, Bethesda, Md. 20014.

Responsibilities of NIH (Specific)

Office of the Director. As suggested by the commentators, for purposes of clarity, the responsibilities of the NIH Director have been grouped under a specific heading in the PRG-NIH entitled "Office of the Director, NIH." These responsibilities (many of which are mentioned in Parts I, II, and III of the PRG-NIH, and are repeated again in Part IV) include: final interpretation of the Guidelines; revision and amendment of the Guidelines; certification of new host-vector systems; promulgating and amending a list of classes of recombinant DNA molecules to be exempt from the Guidelines; permitting specific exceptions to the Prohibitions in the Guidelines; approving changes in containment levels for specific experiments; designating certain agents which are listed as Class 2 agents, as Class 1 agents for the purposes of the Guidelines; and overseeing the implementation of the Guidelines. For many of the responsibilities, appropriate notice and opportunity for public comment will be provided.

Recombinant Advisory Committee. At the hearing in 1976, many commentators made suggestions concerning the structure, function, and scope of responsibility of the RAC. Comments on possible structural mechanisms for decision-making included suggestions that there be a scientific and technical committee and a general advisory public policy committee. It was also suggested that the scientific committee include scientists who are to actively engaged in recombinant DNA research, and that the public policy committee have a broad scientific and public representation. In response to those suggestions in 1976, the roles of the RAC and the NIH Advisory Committee to the Director (DAC) were spelled out. The RAC responsibility has been primarily a scientific and technical one with recommendations for revisions of the Guidelines reviewed by the DAC, the public advisory group. In the main, that process has worked well over the past 1½ years and its structure is maintained in the PRG-NIH.

I am acutely aware of the need for broad scientific representation on the RAC, and I have carefully considered these needs in the selection of new members. The emphasis has been to ensure relevant scientific representation. It is absolutely essential that this committee have the technical expertise necessary to develop, modify, and interpret the Guidelines based on scientific evidence. Additonal representatives have been added from scientific disciplines, such as botany, to ensure a broad scientific overview. As a bridge

between the scientific and the public policy implications, public members now serve on the RAC and additional public members may be added. Current public members are Dr. Emmette S. Redford, Ashbel Smith Professor of Governement and Public Affairs, Lyndon B. Johnson School of Public Affairs, University of Texas at Austin; and Dr. LeRoy Walters, Director, Center for Bioethics, Kennedy Institute, Georgetown University. Both have served the public interest well and have done a superb job as have all members of the committee. The task for all RAC members has been enormous and their work and spirit of cooperation have been exemplary.

In order to ensure fairness, and sensitivity to the public commentators, solicitation of nominations for openings on the RAC will be in accord with the recommendations of the NIH Grants Peer Review Study Team concerning announcements of vacancies on committees. Thus, NIH will publish, periodically, an announcement of upcoming vacanices on the RAC with instructions on how to submit nominations. By this means I will be able to consider carefully a wide spectrum of nominations and assure appropriate representation suited to the needs of this committee.

One commentator suggested that representatives from Federal agencies serve on the RAC. Several agencies, including the National Science Foundation, the Department of Energy, and the Department of Agriculture, have liaison representatives who come regularly to the RAC meetings and, of course, the Federal Interagency Committee is kept fully informed of the activities of this committee.

It was also recommended by a commentator that the NIH finance the cost of attendance at RAC meetings by interest members of the public. For the present I do not believe such a policy is necessary, especially in light of the responsibilities of the DAC for public oversight where public witnesses may be invited and their expenses paid (as they were at the December 1977 hearing). All RAC meetings are announced in the FEDERAL REGISTER and are open to the public.

In sum, the operations of the RAC have been more clearly detailed in the PRG-NIH. The procedures for the selection of members and the operations of the committee have been, or are in the process of being, formalized for the benefit of the scientific community and the public.

NIH Components. A new section now describes all other functions of the NIH including the responsibilities of the Office of Recombinant DNA Activities (ORDA). Several commentators at the public hearing in 1976 urged that the NIH create an office to coordinate recombinant DNA activities. On the basis of these suggestions, ORDA was created and Dr. William Gartland was named Director. Since its creation, ORDA has done a splendid job in fulfilling very difficult tasks in the implementation of the NIH Guidelines. Dr. Garland, who also serves as Executive Secretary to the RAC, has provided a focus for coordination of activities within the NIH and with institutional biosafety committees.

It is important to note that the responsibility of the NIH peer review groups (e.g., study sections) for an independent assessment of the recombinant DNA research protocols has been eliminated. This responsibility will now solely be that of ORDA in conjunction with the institutional biosafety committee. In the 1½ years of our experience, such review by NIH peer review groups has been found to be unnecessary and an additional burden on these groups.

Several commentators urged new responsibilities for ORDA and additional personnel to fulfill them. Several urged that ORDA be responsible for inspecting and certifying laboratories at the P3 level. At the present time P4 facilities are operating at the Frederick Cancer Research Center in Frederick, Md, and at the NIH in Bethesda, Md; no other P4 facilities for recombinant DNA research are in operation nationally. The NIH has the responsibility under the Guidelines to certify P4 facilities because of their special nature. However, a P3 facility does not require special expertise at a national level; and there is no need for national certification of P3 facilities. As specified, the local institution has responsibility for monitoring and certifying facilities from the P1 to the P3 level and that, indeed, should be a local responsibility.

Several commentators urged greater dissemination of information to the public and scientific community alike. ORDA has a key responsibility for the dissemination of information through the "Recombinant DNA Technical Bulletin." The Bulletin is a new publication that attempts to link investigators involved in recombinant DNA research, both in the United States and abroad, with the advisory groups and organizations active in this area. In light of comments received, the Bulletin will include more information for institutional biosafety committees, as well as for the advisory groups at the national and local levels. It was suggested that ORDA provide advice to state and local governments, and to the most practical extent, ORDA will be available to state and local governments for technical advice. In large part, ORDA serves as a clearinghouse for information related to recombinant DNA activities internationally, nationally, and locally.

Registration and Compliance (General)

Over the past 2 years in the administration of the NIH Guidelines, it has been clear to me that a new section should be added on the general requirements for registration of activities with the NIH, not only for NIH grantees or contractors, but also, on a voluntary basis, for the private sector. Further, in light of the review of HEW policies on the patenting of recombinant DNA research inventions, a section on disclosure of information was also necessary. And finally, as suggested, a section on compliance with the Guidelines was needed. Thus, new sections C and D have been added under "Roles and Responsibilities" covering Registration (including disclosure of information) and Compliance. Many comments on the Guidelines over the past 1½ years and at the public hearing in December 1977 urged that these provisions be added, and in my view, they are necessary in the absence of legislation. Further, if legislation were to pass, these provisions could serve as a model for the regulations to be promulgated on the basis of the legislation. As in 1976, I believe the Guidelines should not become regulations without new legislation specifically mandating this.

Registration and Compliance (Specific)

Section IV-C has been added providing the elements for registration. Other requirements may need to be added; notice will be given of any change in the requirements. All projects subject to the Guidelines must be registered with ORDA. A mechanism for voluntary registration by the private sector has been provided in response to suggestions by private sector representatives. A requirement for registration is that the registrant must agree to abide by the standards of the Guidelines.

Many comments were directed to the protection of proprietary information. A new section outlining the elements for protection of proprietary data has been included in response to these suggestions.

One commentator urged that no patents be granted for recombinant DNA research inventions. Shortly after the release of the Guidelines in 1976, NIH received a letter requesting a review of HEW policies relating to the patenting of recombinant DNA research inventions. The letter prompted NIH to review current patent regulations governing existing institutional patent agreements and to consider how recombinant DNA research inventions should be handled under the terms of those agreements. On the basis of ex-

tensive Department and Interagency Committee review, it was agreed that, at least for the present, recombinant DNA research inventions developed under HEW/NIH support should continue to be administered within current HEW patent agreements. Each agreement, however, would require that licenses could be granted only if the licensee provides assurance of compliance with the physical and biological containment standards set forth in the Guidelines. My decision and analysis on this were released in March 1978. A copy is available from the Office of Recombinant DNA Activities, NIH, Bethesda, Md. 20014. Thus, I do not believe that a restriction of patents in this research area is warranted.

A commentator urged that a system of fines be spelled out. NIH has no authority to impose fines in the absence of new legislation. However, NIH will suspend, limit, or terminate a grant or contract for noncompliance with the Guidelines. A commentator urged that penalty procedures be specified. Should it be necessary to suspend, limit, or terminate a grant, appropriate HEW procedures will be followed.

In sum, Part IV of the Guidelines on Roles and Responsibilities has been substantially revised in response to the suggestions from many commentators. The Guidelines now provide even more opportunity for advice from the local to the national level. The spirit of cooperation and effective oversight will be enhanced by the revised Guidelines both at the local level between the research community and the public and at the national level with Federal agencies, the scientific community, and private sectors.

DONALD S. FREDRICKSON,
Director,
National Institutes of Health.

RECOMBINANT DNA RESEARCH—REVISED GUIDELINES PROPOSED BY THE DIRECTOR, NIH

JULY 1978.

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I. SCOPE OF THE GUIDELINES

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

I-B. *Definition of Recombinant DNA Molecules.* In the context of these guidelines, recombinant DNA molecules are defined as either (i) molecules which are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) DNA molecules that result from the replication of those described in (i) above.

I-C. *General Applicability.* The guidelines are applicable to all recombinant DNA research within the United States or its territories conducted at or sponsored by an institution that receives any support for recombinant DNA research from NIH. This includes research performed directly by NIH.

Any individual receiving support must be associated with or sponsored by an institution which can and does assume responsibilities described in these guidelines.

Once approved at the local level, research may proceed but shall be modified in accordance with the recommendations of the NIH if found not to

comport with requirements of the NIH guidelines.

The guidelines are also applicable to projects done abroad if they are supported by NIH funds. If the host country has established rules for the conduct of recombinant DNA projects, then a certificate of compliance with the host country rules may be submitted to NIH in lieu of compliance with the guidelines. NIH reserves the right to withhold funding if the safety practices to be employed are not reasonably consistent with the NIH guidelines. In any case, a memorandum of understanding and agreement (MUA) shall be submitted to NIH for purposes of registration.

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

I-D-1. Formation of recombinant DNA's derived from the pathogenic organisms classified(*1*) as class 3, 4, or 5(*2*) or from cells known to be infected with such agents, regardless of the host-vector system used.

I-D-2. Deliberate formation of recombinant DNA's containing genes for the biosynthesis of potent toxins (e.g., botulinum or diphtheria toxins; venoms from insects, snakes, etc.).

I-D-3. Deliberate creation by the use of recombinant DNA of a plant pathogen with increased virulence and host range beyond that which occurs by natural genetic exchange.

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

I-D-5. Deliberate transfer of a drug resistance trait to micro-organisms that are not known to acquire it naturally, if such acquisition could compromise the use of a drug to control disease agents in human or veterinary medicine or agriculture.

I-D-6. Large-scale experiments (e.g., more than 10 liters of culture) with organisms containing recombinant DNA's, unless the recombinant DNA's are rigorously characterized and are shown to be free of harmful genes.(*3*)

We differentiate between small- and large-scale experiments with organisms containing recombinant DNA's because the probability of escape from containment barriers normally increases with increasing scale.

Experiments in these categories may be excepted(*4*) from the prohibitions (and will at that time be assigned appropriate levels of physical and biological containment) provided that these experiments are expressly approved by the Director, NIH, on recommendation of the Recombinant DNA Advisory Committee after appropriate notice and opportunity for public comment. In making such exceptions, weight will be given both to scientific and societal benefits and to potential risks.

I-E. *Exemptions.* It must be emphasized that the following exemptions(*4*) are not meant to apply to experiments described in the section I-D as being prohibited.

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

I-E-1. Those that are not in organisms or viruses.(*5*)

I-E-2. Those that consist entirely of DNA segments from a single nonchromosomal or viral DNA sources, though one or more of the segments may be a synthetic equivalent.

I-E-3. Those that consist entirely of DNA from a prokaryotic host, including its indigenous plasmids or virsuses, when propagated only in that host (or closely related strain of the same species); also those that consist entirely of DNA from a eukaryotic host, including its chloroplasts, mitochondria, or plasmids (but excluding viruses), when propagated only in that host (or a closely related strain of the same species).

I-E-4. Certain specified recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the Director, NIH, on the recommendation of the Recombinant DNA Advisory Committee, after appropriate notice and opportunity for public comment. Certain classes are exempt as of publication of these revised guidelines. The list in appendix A. An updated list may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Md. 20014.

I-E-5. Other classes of recombinant DNA molecules if the Director, NIH, on the recommendation of the Recombinant DNA Advisory Committee, after appropriate notice and opportunity for public comment, finds that they do not present a significant risk to health or the environment.

I-F. *General Definitions.* The following terms, which are used throughout these guidelines, are defined as follows:

I-F-1. "DNA" means deoxyribo- nucleic acid.

I-F-2. "Recombinant DNA molecule" means either (i) molecules which are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) DNA molecules which result from the replication of a molecule described in (i) above.

I-F-3. "Director" means the Director of the National Institutes of Health (NIH) and any other officer or employee of NIH to whom authority has been delegated.

I-F-4. "Institution" means any public or private entity (including Federal, State, and local government agencies).

I-F-5. "Memorandum of Understanding and Agreement" or "MUA" means an institution's certification to NIH that the project will comply with the guidelines. See appendix C regarding the form and contents of an MUA.

I-F-6. "Institutional Biosafety Committee" or "IBC" is discussed in detail in section IV-A-2.

I-F-7. "Area Biosafety Committee" or "ABC" means that in special circumstances, in consultation with the NIH Office of Recombinant DNA Activities, an Area Biosafety Committee may be formed, composed of members from the institution and other organizations beyond its own staff, as an alternative to an IBC when additional expertise outside the institution is needed for the indicated reviews.

I-F-8. "Recombinant DNA Advisory Committee" or "RAC" means the public advisory committee that shall advise the Secretary, Assistant Secretary for Health, and the Director of the National Institutes of Health, concerning recombinant DNA research. The RAC shall consist of members who shall be selected from persons knowledgeable in the fields of recombinant DNA technology, biological safety, and community interests. Nominations for the RAC may be submitted to ORDA and will be considered in accordance with established nomination procedures for NIH peer review groups.

I-F-9. "NIH Office of Recombinant DNA Activities" or "ORDA" means the office within NIH with responsibility to (i) review and coordinate all activities of NIH related to the guidelines; (ii) foster the interrelationships between NIH and other Government agencies, private foundations, professional societies and industry, in order to assure coordination of activities; (iii) promote international cooperation; (iv) review the composition of Institutional Biosafety Committees; (v) review MUA's; (vi) develop registries of activities related to recombinant DNA research (laboratories, projects, new containment facilities, etc.); and (vii) prepare regular reports.

II. Containment

Effective biological safety programs have been operative in a variety of laboratories for many years. Considerable information therefore already exists for the design of physical containment facilities and the selection of laboratory procedures applicable to organisms carrying recombinant DNA's. (*6-19*) The existing programs rely upon mechanisms that, for convenience, can

be divided into two categories: (i) a set of standard practices that are generally used in microbiological laboratories, and (ii) special procedures, equipment, and laboratory installations that provide physical barriers which are applied in varying degrees according to the estimated biohazard.

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

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

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

II-A. *Standard Practices and Training.* The first principle of containment is a strict adherence to good microbiological practices. (*6-15*) Consequently, all personnel directly or indirectly involved in experiments on recombinant DNA's must receive adequate instruction. This should as a minimum include instruction in aseptic techniques and in the biology of the organisms used in the experiments, so that the potential biohazards can be understood and appreciated.

Any research group working with agents with a known or potential biohazard should have an emergency plan which describes the procedures to be followed if an accident contaminates personnel or the environment. The principal investigator must insure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan. If a research group is working with a known pathogen for which an effective vaccine is available, all workers should be immunized. Serological monitoring, where appropriate, should be provided.

II-B. *Physical Containment Levels.* The objective of physical containment is to confine organisms containing novel recombinant DNA molecules, and thus to reduce the potential for exposure of the laboratory worker, persons outside of the laboratory, and the environment to organisms containing novel recombinant DNA molecules. Physical containment is achieved through the use of laboratory practices, containment equipment, and special laboratory design. Emphasis is placed on primary means of physical containment which are provided by laboratory practices and containment equipment. Special laboratory design provides a secondary means of protection against the accidental release of organisms outside the laboratory or to the environment. Special laboratory design is used primarily in facilities in which experiments of moderate to high potential hazard are performed.

Combinations of laboratory practices, containment equipment, and special laboratory design can be made to achieve different levels of physical containment. Four levels of physical containment, which are designated as P1, P2, P3, and P4, are described. It should be emphasized that the descriptions and assignments of physical containment detailed below are based on existing appproaches, to containment of pathogenic organisms. For example, the "Classification of Etiologic Agents on the Basis of Hazard," (7) prepared by the Center for Disease Control, describes four general levels which roughly correspond to our descriptions for P1, P2, P3, and P4; and the National Cancer Institute describes three levels for research on oncogenic viruses which roughly correspond to our P2, P3, and P4 levels.(8)

It is recognized that several different combinations of laboratory practices, containment equipment, and special laboratory design may be appropriate for containment of specific research activities. The guidelines, therefore, allow alternative selections of primary containment equipment within facilities that have been designed to provide P3 and P4 levels of physical containment. The selection of alternative methods of primary containment is dependent, however, on the level of biological containment provided by the host-vector system used in the experiment. Consideration will also be given by the Recombinant DNA Advisory Committee to other combinations which achieve an equivalent level of containment. Additional material on physical containment for plant host-vector systems is found in sections III-C-3 and III-C-4.

II-B-1. *P1 Level.*

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

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

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

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

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

II-B-1-a-(5). Eating, drinking, smoking, and storage of foods are not permitted in the working area.

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

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

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

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

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

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

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

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

II-B-2. *P2 Level.*

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

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

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

II-B-2-a-(3). All biological wastes shall be decontaminated before disposal. Other contaminated materials such as glassware, animal cages, and labora-

tory equipment shall be decontaminated before washing, reuse, or disposal.

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

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

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

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

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

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

II-B-2-a-(10). *Children under 12 years of age shall not enter the laboratory.

II-B-2-a-(11). *The universal biohazard sign shall be posted on all laboratory access doors when experiments requiring P2 containment are in progress. Freezers and refrigerators used to store organisms containing recombinant DNA molecules shall also be posted with the universal biohazard sign.

II-B-2-a-(12). An insect and rodent control program shall be instituted.

II-B-2-a-(13). *The use of laboratory gowns, coats, or uniforms is required. Laboratory clothing shall not be worn to the lunch room or outside of the building in which the laboratory is located.

II-B-2-a-(14). *Animals not related to the experiment shall not be permitted in the laboratory.

II-B-2-a-(15). Use of the hypodermic needle and syringe shall be avoided when alternative methods are available.

II-B-2-a-(16). *The laboratory shall be kept neat and clean.

II-B-2-a-(17). *Experiments of lesser biohazard potential can be carried out concurrently in carefully demarcated areas of the same laboratory.

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

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

II-B-3. *P3 Level.*

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

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

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

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

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

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

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

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

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

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

II-B-3-a-(10). Children under 12 years of age shall not enter the laboratory.

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

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

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

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

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

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

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

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

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

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

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

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

II-B-3-b-(2). *Laboratory animals held in a P3 area shall be housed in partial containment caging systems such as Horsfall units, open cages placed in ventilated enclosures, solid-wall-and-bottom cages covered by filter bonnets, or solid-wall and

*Denotes laboratory practices, containment equipment, or special laboratory design which were not required at the next lower level of containment.

bottom-cages placed on holding racks equipped with ultraviolet radiation lamps and reflectors. (NOTE: Conventional caging systems may be used, provided that all personnel wear appropriate personal protective devices. These shall include at a minimum wraparound gowns, head covers, gloves, shoe covers, and respirators. All personnel shall shower on exit from areas where these devices are required.)

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

Table I

COMBINATIONS OF CONTAINMENT SAFEGUARDS

Classification of experiment according to Guidelines		Alternate combinations of physical and biological containment			
		Physical Containment			
Physical containment	Biological* containment	Laboratory design specified for:	Laboratory practices specified for:	Containment equipment specified for:	Biological containment
P3	HV3	P3	P3	P3	HV3
P3	HV3	P3	P3	P4	HV2
P3	HV2	P3	P3	P3	HV2
P3	HV2	P3	P3	P2	HV3
P3	HV2	P3	P3	P4	HV1
P3	HV1	P3	P3	P3	HV1
P3	HV1	P3	P3	P2	HV2

*See Section II-D for description of biological containment.

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

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

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

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

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

II-B-3-c-(5). *Laboratory doors shall be self-closing.

II-B-3-c-(6). *An autoclave for sterilization of wastes and contaminated materials shall be available in the same building (and preferably within the controlled laboratory area) in which organisms containing recombinant DNA molecules are used.

II-B-3-c-(7). *An exhaust air ventilation system shall be provided. This system shall be balanced so that the direction of airflow is from the controlled access area into the laboratory environment. The exhaust air shall not be recirculated to any other areas of the building. Recirculation of air within the laboratory room, however, may be provided. The exhaust air from the laboratory shall be discharged to the outdoors so that it is dispersed clear of occupied buildings and air intakes. the exhaust air from the laboratory can be discharged to the outdoors without filtration or other treatment.

II-B-3-c-(8). *The treated exhaust air from class I and class II biological safety cabinets (*20*) may be discharged either directly to the laboratory or to the outdoors. The treated exhaust air from a class III cabinet shall be discharged directly to the outdoors. If the treated exhaust air from these cabinets is to be discharged to the outdoors through a building exhaust air system, it shall be connected to this system so as to avoid any interference with the air balance of the cabinet or building exhaust air system.

II-B-4. *P4 Level.*

II-B-4-a. *Laboratory Practices.*

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

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

II-B-4-a-(3). All biological wastes shall be decontaminated before disposal. Other contaminated materials such as glassware, animal cages, and laboratory equipment shall be decontaminated before washing, reuse, or disposal.

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

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

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

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

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

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

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

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

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

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

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

II-B-4-a-(15). *The universal biohazard sign is required on the P4 facility access doors and all interior doors to individual laboratory rooms where experiments are conducted.

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

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

II-B-4-a-(18). Use of the hypodermic needle and syringe shall be avoided when alternate methods are available.

II-B-4-a-(19). The laboratory shall be kept neat and clean.

II-B-4-a-(20). *If experiments involving other organisms which require lower levels of containment are to be conducted in the P4 facility concurrently with experiments requiring P4-level containment, they shall be conducted in accordance with all P4-level laboratory practices specified in this section.

II-B-4-b. *Containment Equipment.*

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

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

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

Table II

COMBINATIONS OF CONTAINMENT SAFEGUARDS

Classification of experiment according to Guidelines		Alternate combinations of physical and biological containment			
		Physical containment			
Physical containment	Biological* containment	Laboratory design specified for:	Laboratory practices specified for:	Containment equipment specified for:	Biological containment
P4	HV1	P4	P4	P4	HV1
P4	HV1	P4	P4	P3	HV2

* See Section II-D for description of biological containment.

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

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

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

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

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

II-B-4-c-(5). *Foot-operated water fountains are permitted in the corridors of the P4 facility. The water service provided to the fountain shall be protected from the water services to the laboratory areas of the P4 facility.

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

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

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

II-B-4-c-(9). *All liquid effluents from the P4 facility shall be collected and decontaminated before disposal. Liquid effluents from biological safety cabinets and laboratory sinks shall be

sterilized by heat. Liquid effluents from the shower and hand washing facilities may be inactivated by chemical treatment. HEPA filters shall be installed in all effluent drain vent lines.

II-B-4-c-(10). *An individual supply and exhaust-air ventilation system shall be provided for the P4 facility. The system shall maintain pressure differentials and directional airflow as required to assure inflow from areas outside the P4 facility toward areas of highest potential risk within the facility. The system shall be designed to prevent the reversal of airflow. The system shall sound an alarm in the event of system malfunction.

II-B-4-c-(11). *Recirculation of air within individual laboratories of the P4 facility is permissible if this air is filtered by a HEPA filter.

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

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

II-B-4-c-(14). *A specially designed suit area may be provided in the facility. Personnel who enter this area shall wear a one-piece positive-pressure suit that is ventilated by a life-support system. The life-support system shall be provided with alarms and emergency backup air. Entry to this area is through an air-lock fitted with airtight doors. A chemical shower area shall be provided to decontaminate the surfaces of the suit before removal. The exhaust air from the suit area shall be filtered by two sets of HEPA filters installed in series, and a duplicate filtration unit and exhaust fan shall be provided. The air pressure within the suit area shall be less than that in any adjacent area. An emergency lighting system, communication systems, and power source shall be provided.

The internal shell of the suit area shall be airtight. A doubledoor autoclave shall be provided for sterilization of all waste materials to be removed from the suit area.

II-C. *Shipment.* Recombinant DNA when contained in an organism or virus shall be shipped in compliance with the requirements issued by the U.S. Public Health Service (section 72.25 of Part 72, Title 42, Code of Federal Regulations), Department of Transportation (section 173.387(b) of Part 173, Title 49, Code of Federal Regulations), and the Civil Aeronautics Board (C.A.B. No. 82, Official Air Transport Restricted Articles Tariff No. 6-D) for shipment of etiologic agents.

The packaging and shipment of organism and viruses containing recombinant DNA molecules shall be in compliance with all requirements specified in subparagraphs (1)-(5) of paragraph (c), "Transportation; etiologic agents subject to additional requirements," of section 72.25 of Part 72, Title 42, Code of Federal Regulations. Subparagraph (6) of paragraph (c) of section 72.25 of Part 72, Title 42, Code of Federal Regulations shall apply to the shipment of all viable host and vector organisms which require P4 physical containment.

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

II-D. *Biological containment.*

II-D-1. *Levels of biological containment.* In consideration of biological containment, the vector (plasmid, organelle, or virus) for the recombinant DNA and the host (bacterial, plant, or animal cell) in which the vector is propagated in the laboratory will be considered together. Any combination of vector and host which are to provide biological containment must be constructed so that the following types of "escape" are minimized: (i) Survival of the vector in its host outside the laboratory, and (ii) transmission of the vector from the propagation host to other nonlaboratory hosts.

The following levels of biological containment (HV, or *H*ost-*V*ector, systems) for prokaryotes will be established; specific criteria will depend on the organisms to be used. Eukaryotic host-vector systems are considered in Part III.

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

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

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

II-D-1-b. *HV2.* These are host-vector systems shown to provide a high level of biological containment as demonstrated by data from suitable tests performed in the laboratory. Escape of the recombinant DNA either via survival of the organisms or via transmission of recombinant DNA to other organisms should be less than $1/10^8$ under specified conditions. *Specific systems:*

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

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

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

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

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

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

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

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

II-D-2. *Certification of host-vector systems.*

II-D-2-a. *Responsibility.* HV1 systems other than *E. coli* K-12, and HV2 and HV3 host-vector systems may not

be used unless they have been certified by the NIH. Application for certification of a host-vector system is made by written application to the Office of Recombinant DNA Activities (ORDA), National Institutes of Health, Bethesda, Md. 20014.

When appropriate, the proposed host-vector system will be reviewed by the NIH Recombinant DNA Advisory Committee (RAC). This may first involve review of the data on construction, properties, and testing of the proposed host-vector system by a subcommittee composed of one or more members of the RAC and other individuals chosen because of their expertise in evaluating such data. The committee will then evaluate the report of the subcommittee and any other available information at a regular meeting.

When new host-vector systems are certified, notice of the certification will be sent to the applicant and to all IBCs and will be published in the "recombinant DNA Technical Bulletin." Copies of a list of all currently certified host-vector systems may be obtained from ORDA at any time.

NIH may at any time rescind the certification of any host-vector system. If certification of a host-vector system is rescinded, investigators may be asked to transfer cloned DNA into a different system.

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

II-D-2-b. *Data to be Submitted for Certification*

II-D2-b-(1). *HV1 Systems Other than E. Coli K-12.* The following types of data should be submitted, modified as appropriate for the particular system under consideration: (i) A description of the organism and vector; the strain's natural habitat and growth requirements; the range of organisms with which this organism normally exchanges genetic information and what sort of information is exchanged; any relevant information on its pathogenicity or toxicity. (ii) A description of the history of the particular strains and vectors to be used, including data on any mutations which render this organism less able to survive or transmit genetic information. (iii) A general description of the range of experiments contemplated, with emphasis on the need for developing such an HV1 system.

I-D-2-b-(2). *HV2 Systems.* Investigators planning to request HV2 certification for host-vector systems can obtain instructions from ORDA concerning data to be submitted. In general, the following types of data are required: (i) Description of construction steps, with indication of source, properties, and manner of introduction of genetic traits. (ii) Quantitative data on the stability of genetic traits that contribute to the containment of the system. (iii) Data on the survival of the host-vector system under non-permissive laboratory conditions designed to represent the relevant natural environment. (iv) Data on transmissibility of the vector and/or a cloned DNA fragment under both permissive and non-permissive conditions. (v) Data on all other properties of the system which affect containment and utility, including information on yields of phage or plasmid molecules, ease of DNA isolation, and ease of transfection or transformation. (vi) In some cases, the investigator may be asked to submit data on survival and vector transmissibility from experiments in which the host-vector is fed to laboratory animals (e.g., rodents). Such *in vivo* data may be required to confirm the validity of predicting *in vivo* survival on the basis of *in vitro* experiments.

Data must be submitted in writing to ORDA. Ten to twelve weeks are normally required for review and circulation of the data prior to the meeting at which such data can be considered by the NIH Recombinant DNA Advisory Committee (RAC). Investigators are encouraged to publish their data on the construction, properties, and testing of proposed HV2 systems prior to consideration of the system by the RAC and its subcommittee. More specific instructions concerning the type of data to be submitted to NIH for proposed EK2 systems involving either plasmids or bacteriopnage 0 in *E. coli* K-12 are available from ORDA

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

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

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

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

NIH may also distribute certified HV1 host-vector systems.

III. Containment Guidelines for Covered Experiments

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

Containment guidelines for permissible experiments are given in part III. Changes in these levels for specific experiments (or the assignment of levels to experiments not explicitly considered here) may be expressly approved by the Director, NIH, on the recommedation of the Recombinant DNA Advisory Committee (RAC).

III-A. *Classification of Experiments Using the E. coli K-12 Host-Vector Systems.* Most recombinant DNA experiments currently being done employ *E. coli* K-12 host-vector systems. These are the systems for which we have the most experience and knowledge (i) regarding the effectiveness of biological containment provided by existing hosts and vectors, and (ii) necessary for the construction of more effective biological barriers. We therefore consider DNA recombinants in *E. coli* K-12 before proceeding to other host-vector systems. The levels of biological containment for *E. coli* K-12 systems

are designated EK1, EK2, and EK3 in ascending order.

It has been necessary, throughout this section, to use words and phrases such as "purified" or "rigorously characterized." In the text such terms are marked with footnote reference numbers. These footnotes (part V) define more fully what these terms denote.

In the following classification of containment criteria for different kinds of recombinant DNA's, the stated levels of physical and biological containment are minimal for the experiments designated. The use of higher levels of biological containment (EK3 > EK2 > EK1) is encouraged if they are available and equally appropriate for the purposes of the experiment.

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

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

III-A-a-(1). *Primates.* P2 physical containment + and EK2 host-vector. Any lowering of containment below these levels (i.e., for purified DNA or characterized clones) cannot be made solely by an institutional biosafety committee but requires NIH approval.

III-A-1-a-(2). *Other Mammals.* P2 physical containment + an EK2 host-vector.

III-A-1-a-(3). *Birds.* P2 physical containment + an EK2 host-vector.

III-A-1-a-(4). *Cold-Blooded Vertebrates.* P2 physical containment + an EK1 host-vector or P1 + EK2. If the eukaryote is known to produce a potent polypeptide toxin,[34] the containment shall be increased to P3 + EK2.

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

III-A-1-a-(5)-(a). Species that are known to produce a potent polypeptide toxin(*34*) that acts in vertebrates, or are known pathogens listed in Class 2,(*1*) or are known to carry such pathogens must use P3 physical containment + an EK2 host-vector. When the potent toxin is not a polypeptide and is likely not to be the product of closely linked eukaryote genes, containment may be reduced to P3 + EK1 or P2 + EK2. Species that produce potent toxins that affect invertebrates of plants but not vertebrates require P2 + EK2 or P3 + EK1. Any species that has a demonstrated capacity for carrying particular pathogenic microorganisms is included in this group, unless the organisms used as the source of DNA have been shown not to contain those agents, in which case they may be placed in the following group.

III-A-1-a-(5)-(b). The remainder of the species in this class including plant pathogenic or symbiotic fungi that do not produce potent toxins: P2 + EK1 or P1 + EK2. However, any insect in this group must be either (i) grown under laboratory conditions for at least 10 generations prior to its use as a source of DNA, or (ii) if caught in the wild, must be shown to be free of dissease-causing microorganisms or must belog to a species that does not carry microorganisms causing disease in vertebrates or plants. If these conditions cannot be met, experiments must be done under P3 + EK1 or P2 + EK2 containment.

III-A-1-a-(6). *Plants.* P2 physical containment + an EK1 host-vector or P1 + EK2. If the plant source makes a potent polypeptide toxin,(*34*) the containment must be raised to P3 physical containment + an EK2 host-vector. When the potent toxin is not a polypeptide and is likely not to be the product of closely linked plant genes, containment may be reduced to P3 + EK1 or P2 + EK2.

III-A-1-b. *Prokaryotic DNA Recombinants.*

III-A-1-b-(1). *Prokaryotes That Exchange Genetic Information(35) with E. Coli.* It is expected that many of the prokaryotes that exchange genetic information with *E. coli* by known physiological processes will be exempted from these Guidelines by appearing on the 'list of exchangers' (see Section I-E-4).

For those not on the list, the containment levels are P1 physical containment + an EK1 host-vector. In fact, experiments in this category may be performed with any *E. coli* K-12 vector (e.g., conjugtive plasmids). However, for prokaryotes that are classified(*1*) as Class 2 the containment levels are P2 + EK1.

III-A-1-b-(2). *Prokaryotes That Do Not Exchange Genetic Information With E. Coli.* P2 physical containment + an EK1 host-vector, or P1 + EK2, except for DNA from Class 2 agents,(*1*) which require P3 + EK2.

III-A-2. *Plasmids, Bacteriophages, and Other Viruses.* Recombinants formed between a vector and some other plasmid or virus DNA have in common the potential for acting as double vectors because of the replication functions in these DNA's. The containment conditions given below apply only to propagation of the DNA recombinants in *E. coli* K-12 hosts. They do not apply to other hosts in which the recombinants may be able to replicate as a result of functions provided by the DNA inserted into the EK vectors. These are considered under other host-vector systems.

III-A—2—a. *Viruses of Eukaryotes.* (summary given in Table III).

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

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

III-A-2-a-(1)-(a)-(*1*). *Adeno-Associated Viruses, Minute Virus of Mice, Mouse Adenovirus (strain FL), and Plant Viruses.* P1 physical containment + and EK1 host-vector shall be used for DNA recombinants produced with (i) the whole viral genome, (ii) subgenomic DNA segments, or (iii) purified cDNA copies of viral mRNA.(*37*).

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

III-A-2-a-(1)-(a)-(*2*)-(*a*). P1 physical containment + an EK1 host-vector shall be used for purified subgenomic DNA segments.

III-A-2-a-(1)-(a)-(*2*)-(*b*). P2 physical containment + an EK2 host-vector or P3 + EK1 shall be used for DNA for recombinants produced with the whole viral genome.

III-A-2-a-(1)-(a)-(*2*)-(*c*). P2 physical containment + an EK1, shall be used for DNA recombinants derived from purified cDNA copies of viral mRNA.

III-A-2-a-(1)-(a)-(*3*). *Other Non transforming Members of Presently Classified Viral Families.(36)*

III-A-2-a-(1)-(a)-(*3*)-(*a*). P1 physical containment + an EK1 host-vector shall be used for (i) DNA recombinants produces with purified subgenomic DNA segments or (ii) purified cDNA copies of viral mRNA.(*37*)

III-A-2-a-(1)-(a)-(*3*)-(*b*). P1 physical containment + an EK1 host and a vector certified for use in an EK2 system shall be used for DNA recombinants produced with the whole viral genome.

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

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

III-A-2-a-(1)-(b)-(*1*)-(*a*). P1 physical containment + an EK1 host-vector shall be used for DNA recombinants produced with purified nontransforming subgenamic DNA segments.(*38*)

III-A-2-a-(1)-(b)-(*1*)-(*b*). P2 physical containment + an EK1 host and a vector certified for use in an EK2 system or P3 + EK1 shall be used for (i) DNA recombinants produced with purified subgenamic DNA segments containing an entire transforming gene or (ii) purified cDNA copies of viral mRNA.(*37*)

III-A-2-a-(1)-(b)-(*1*)-(c). P3 physical containment + an EK1 host-vector or P2 + EK2 shall be used for DNA recombinants produced with the whole viral genome.

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

III-A-2-a-(1)-(b)-(*2*)-(*a*). P1 physical containment + an EK1 host-vector

shall be used for DNA recombinants produced with purified nontransforming subgenamic DNA segments.(*38*)

III-A-2-a-(1)-(b)-(*2*)-(*b*). P2 physical containment + an EK1 host and a vector certified for use in an EK2 system or P3 + EK1 shall be used for (i) DNA recombinants produced with the whole viral genome, (ii) purified subgenamic DNA segments containing an entire transforming gene, or (iii) purified cDNA copies of viral mRNA.(*37*)

III-A-2-a-(2). *DNA Transcripts of RNA Viruses.*

III-A-2-a-(2)-(a). *Retroviruses.*

III-A-2-a-(2)-(a)-(*1*). *Gibbon Ape, Woolly Monkey, Feline Leukemia and Feline Sarcoma Viruses.*(*39*)

III-A-2-a-(2)-a-(*1*)-(*a*). P1 physical containment + an EK1 host-vector shall be used for DNA recombinants produced with purified nontransforming subgenamic DNA segments.(*38*)

III-A-2-a-(2)-(*1*)-(*b*). P2 physical containment + an EK1 host and a vector certified for use in an EK2 system, or P3 + EK1, shall be used for DNA recombinants produced with purified subgenamic DNA segments(*38*) containing an entire transforming gene.

III-A-2-a-(2)-(*1*)-(c). P2 physical containment + an EK2 host-vector or P3 + EK1 shall be used for DNA recombinants produced with (i) the whole viral genome or (ii) purified cDNA copies of viral mRNA.(*37*)

III-A-2-a-(2)-(a)-(*2*). *Other Members of the Family Retroviridiae.*(*36*)

III-A-2-a-(2)-(a)-(*2*)-(*a*). P1 physical containment + an EK1 host-vector shall be used for DNA recombinants produced with purified nontransforming subgenamic DNA segments.(*38*)

III-A-2-a-(2)-(a)-(*2*)-(*b*). P2 physical containment + an EK1 host and a vector certified for use in an EK2 system or P3 + EK1 shall be used for DNA recombinants produced with (i) purified subgenamic DNA segments containing an entire transforming gene, (ii) the whole viral genome, or (iii) purified cDNA copies of viral mRNA.(*37*)

III-A-2-a-(2)-(b). *Negative Strand RNA Viruses.* P1 physical containment + an EK1 host-vector shall be used for DNA recombinants produced with (i) cDNA copies of the whole genome, (ii) subgenomic cDNA segments, or (iii) purified cDNA copies of viral mRNA.(*37*)

III-A-2-a-(2)-(c). *Plus-Strand RNA Viruses.*

III-A-2-a-(2)-(c)-(*1*). *Types 1 and 2 Sabin Poliovirus Vaccine Strains and Strain 17D (Theiler) of Yellow Fever Virus.* P1 physical containment + and EK1 host-vector shall be used for DNA recombinants produced with (i) cDNA copies of the whole viral genome, (ii) subgenomic cDNA segments, or (iii) purified cDNA copies of viral mRNA.(*37*)

III-A-2-a-(2)-(c)-(*2*). *Other Plus-Strand RNA Viruses Belonging to Presently Classified Viral Families.*(*36*)

Table III

Recommended Containment for Cloning of Viral DNA or cDNA in E. coli K-12 Host-Vector Systems
(See text for full details)

Virus class	Type of viral DNA segment to be cloned				
	Subgenomic[38]		Genomic		cDNA from viral mRNA[37]
	Nontransforming segment	Segment containing an entire transforming gene	Non-segmented genome	Segmented genome	
DNA					
Nontransforming viruses					
AAV, MVM, Mouse Adeno (Strain FL)	P1 + EK1		P1 + EK1		P1 + EK1
Plant Viruses	P1 + EK1		P1 + EK1		P1 + EK1
Hepatitis B	P1 + EK1		P2 + EK2 or P3 + EK1		P2 + EK1CV[40] or P3 + EK1
Other	P1 + EK1		P1 + EK1CV[40]		P1 + EK1
Transforming Viruses					
Herpes Saimiri, H. Ateles and EBV[39]	P1 + EK1	P2 + EK1CV[40] or P3 + EK1	P2 + EK2 or P3 + EK1		P2 + EK1CV[40] or P3 + EK1
Other	P1 + EK1	P2 + EK1CV[40] or P3 + EK1	P2 + EK1CV[40] or P3 + EK1		P2 + EK1CV[40] or P3 + EK1
RNA					
Retroviruses					
Gibbon Ape, Woolly Monkey FeLV and FeSV[39]	P1 + EK1	P2 + EK1CV[40] or P3 + EK1	P2 + EK2 or P3 + EK1		P2 + EK2 or P3 + EK1
Other	P1 + EK1	P2 + EK1CV[40] or P3 + EK1	P2 + EK1CV[40] or P3 + EK1		P2 + EK1CV[40] or P3 + EK1

Table III

Recommended Containment for Cloning of Viral DNA or cDNA in E. coli K-12 Host-Vector Systems
(See text for full details)

Virus class	Type of viral DNA segment to be cloned				
	Subgenomic[38]		Genomic		cDNA from viral mRNA[37]
	Nontransforming segment	Segment containing an entire transforming gene	Nonsegmented genome	Segmented genome	
Negative Strand RNA	P1 + EK1		P1 + EK1	P1 + EK1	P1 + EK1
Plus Strand RNA					
Types 1 and 2 Sabin Polio, 17D Yellow Fever Vaccine Strains	P1 + EK1		P1 + EK1		P1 + EK1
Other	P1 + EK1		P2 + EK1CV[40] or P3 + EK1		P2 + EK1CV[40] or P3 + EK1
Double Stranded RNA	P1 + EK1			P1 + EK1	P1 + EK1
Plant Viruses + Viroids	P1 + EK1		P1 + EK1	P1 + EK1	P1 + EK1
Intracellular Viral DNA	See text	See text	See text		

III-A-2-a-(2)-(c)-(2)-(*a*). P1 physical containment + an EK1 host-vector shall be used for DNA recombinants produced with purified subgenomic cDNA segments.(*38*)

III-A-2-a-(2)-(c)-(2)-(*b*). P2 physical containment + an EK1 host and a vector certified for use in an EK2 system of P3+EK1 shall be used for DNA recombinants produced with (i) cDNA copies of the whole genome, or (ii) purified cDNA copies of viral mRNA.(*37*)

III-A-2-a-(2)-(d). *Double-Stranded Segmented RNA Viruses.* P1 physical containment + an EK1 host-vector shall be used for DNA recombinants produced with (i) mixtures of subgenomic cDNA segments, (ii) a specific subgenomic cDNA segment, or (iii) purified cDNA copies of viral mRNA. (*37*)

III-A-2-a-(2)-(e). *RNA Plant Viruses and Plant Viroids.* P1 physical containment + an EK1 host-vector shall be used for DNA recombinants produced with (i) cDNA copies of the whole viral genome, (ii) subgenomic cDNA segments, or (iii) purified cDNA copies of viral mRNA.(*37*)

III-A-2-a-(3). *Intracellular Viral DNA.* Physical and biological containment specified for shotgun experiments with eukaryotic cellular DNA [see section III-A-(1)-(a)] shall be used for DNA recombinants produced with integrated viral DNA or viral genomes present in infected cells.

III—A-2-b. *Eukaryotic Organelle DNA's.* P2 physical containment + an EK1 host-vector, or P1+EK2, for mitochondrial or chloroplast DNA from eukaryotes when the organelle DNA has been obtained from isolated organelles. Otherwise, the conditions given for shotgun experiments apply.

III-A-2-c. *Prokaryotic Plasmid and Phage DNA's.* The containment levels required for shotgun experiments with DNA from prokaryotes apply to their plasmids or phages.

III-A-3. *Lowering of Containment Levels for Characterized or Purified DNA Preparations and Clones.* Many of the risks which might conceivably arise from some types of recombinant DNA experiments, particularly shotgun experiments, would result from the inadvertent cloning of a harmful sequence. Therefore, in cases where the risk of inadvertently cloning the "wrong" DNA is reduced by prior enrichment for the desired piece, or in which a clone, made from a random assortment of DNA's, has been purified and the absence of harmful sequences established, the containment conditions for further work may be reduced. The following section outlines the mechanisms for such reductions.

III-A-3-a. *Purified DNA Other than Plasmids, Bacteriophages, and Other Viruses.* The formation of DNA recombinants from cellular DNA's that have been purified (*41*) and which are free of harmful genes(*3*) can be carried out under lower containment conditions than used for the corresponding shotgun experiment. (*42*) The containment may be decreased one step in physical containment (P4 → P3 → P2 → P1) while maintaining the biological containment specified for the shotgun experiment or one step in biological containment (EK3 → EK2 → EK1) while maintaining the specified physical containment. The institutional biosafety committee (IBC) must review and may approve such a reduction. The IBC must notify the NIH Office of Recombinant DNA Activities (ORDA) in writing of all such actions. IBC approval is sufficient for such a reduction except for (i) primate DNA which also requires prior NIH approval [see section III-A-1-a-(1)] or (ii) any lowering of containment under section III-A-3-a to levels below P1+EK1, which also requires prior NIH approval.

III-A-3-b. *Characterized Clones of DNA Recombinants.* When a cloned DNA recombinant has been rigorously characterized and there is sufficient evidence that it is free of harmful genes, (*3*) experiments involving this recombinant DNA may be carried out under lower containment conditions, as described below.

III-A-3-b-(1). Institutional biosafety committees (IBC's) may give approval for a single-step reduction in physical or biological containment on receipt of evidence of characterization of a clone derived from a shotgun experiment and its probable freedom from harmful genes. The IBC must notify ORDA in writing of all such actions. IBC approval is sufficient for such a reduction except for (i) primate DNA, which requires prior NIH approval [see section III-A-1-a-(1)], or (ii) any lowering of containment under section III-A-3-b to levels below P1+EK1, which also requires prior NIH approval.

III-A-3-b-(2). Reduction of containment levels by more than one step or cases involving primate DNA, or cases involving lowering of containment under section III-A-3-b to levels below P1+EK1, will require prior approval by NIH.

III-B. *Experiments with Other Prokaryotic Host-Vectors.*

III-B-1. *HV1 systems.* Host-vector systems which have been approved as HV1 systems may be used under P2 containment conditions for shotgun experiments with phages, plasmids, and DNA from nonpathogenic prokaryotes which do not produce polypeptide toxins.(*34*)

Other classes of recombinant DNA experiments with these HV1 systems will require prior approval and classification by NIH. Experiments with DNA's from eukaryotes (and their plasmids or viruses) will generally follow the criteria for the corresponding experiments with *E. coli* K-12 host-vectors if the major habitats of the given host-vector overlap those of *E. coli*. The habitats of other host-vector systems should also be considered in relation to containment.

III-B-2. *Return of DNA Segments to Non-HV1 Host of Origin.* Many of the prokaryotes that exchange genetic information with *E. coli* by known physiological processes are expected to be exempt from these Guidelines by appearing on the "list of exchangers" (see Section I-E-4). For a prokaryote which can exchange genetic information (*35*) with *E. coli* under laboratory conditions but which is not on the list (Host A), the following type of experiment may be carried out under P1 conditions without Host A having been approved as an HV1 host: DNA from Host A may be inserted into a vector and propagated in *E. coli* K-12 under P1 conditions. Subsequently, this recombinant DNA may be returned to Host A by mobilization, transformation, or transduction and may then be propagated in Host A in any desired vector under P1 conditions.

For a prokaryote which does not exchange genetic information with *E. coli* (Host B), the following type of experiment may be carried out without Host B having been approved as an HV1 host: DNA from Host B may be inserted into a vector from a certified EK2 host-vector system and propagated in *E. coli* K-12 under the appropriate containment conditions [see section III-A-1-b-(2)]. Subsequently, this recombinant DNA may be returned to Host B and propagated in Host B under P1 conditions.(*43*)

III-C. *Experiments with Eukaryotic Host-Vectors.*

III-C-1. *Vertebrate Host-Vector Systems.* (*44*) (Summary Given in Table IV).

III-C-1-a. *Polyoma Virus.*

III-C-1-a-(1). *Productive Virus-Cell Interactions.*

III-C-1-a-(1)-(a). Defective or intact polyoma virus genomes, with appropriate helper, if necessary, can be used in P2 conditions to propagate DNA sequences:

III-C-1-a-(1)-(a)-(*1*). From bacteria of class 1 or class 2[1] or their phages or plasmids, except for those that produce potent polypeptide toxins; (*34*)

III-C-1-a-(1)-(a)-(*2*). From mice;

III-C-1-a-(1)-(a)-(*3*). From eukaryotic organisms that do not produce potent polypeptide toxins, (*34*) provided the DNA segment is > 99 percent pure.

III-C-1-a-(1)-(b). Defective polyoma genomes, with appropriate helper, if necessary, can be used in P2 conditions for shotgun experiments to propagate DNA sequences from eukaryotic or-

ganisms that do not produce potent polypeptide toxins.(*34*)

III-C-1-a-(1)-(c). Intact virus genomes with appropriate helper, if necessary, can be used in P3 conditions for shotgun experiments to propagate DNA sequences from eukaryotic organisms that do not produce potent polypeptide toxins.(*34*)

III-C-1-a-(1)-(d). Experiments involving the use of defective polyoma virus genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by NIH on a case-by-case basis (*45*) and will be conducted under the recommended physical containment conditions.

III-C-1-a-(2). *Nonproductive Virus-Cell Interactions.* Defective or intact polyoma virus genomes can be used as vectors in P2 conditions to transform nonpermissive cells in culture, provided the inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis.(*45*)

III-C-1-b. *Simian Virus 40.*

III-C-1-b-(1). *Productive Viruses-Cell Interactions.*

III-C-1-b-(1)-(a). SV40 DNA, rendered unconditionally defective by a deletion in an essential gene, with appropriate helper, can be used in P2 conditions to propagate DNA sequences from:

III-C-1-b-(1)-(a)-(*1*). Bacteria of Class 1 or Class 2[1], or their phages or plasmids, except for those that produce potent polypeptide toxins;(*34*)

III-C-1-b-(1)-(a)-(*2*). Uninfected African green monkey kidney cell cultures.

III-C-1-b-(1)-(b). SV40 DNA, rendered unconditionally defective by a deletion in an essential gene, with an appropriate helper, can be used in P3 conditions to propagate DNA sequences from eukaryotic organisms that do not produce potent polypeptide toxins(*34*) (shotgun experiments or purified DNA).

III-C-1-b-(1)-(c). Experiments involving the use of defective SV40 genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by the NIH on a case-by-case basis (*45*) and will be conducted under the recommended physical containment conditions.

III-C-1-b-(2). *Nonproductive Virus-Cell Interactions.* Defective or intact SV40 genomes can be used as vectors in P2 conditions to transform nonpermissive cells in culture, provided the inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis.(*45*)

III-C-1-c. *Human Adeno- viruses 2 and 5.*

III-C-1-c-(1). *Productive Virus-Cell Interactions.*

III-C-1-c-(1)-(a). Human adenoviruses 2 and 5, rendered unconditionally defective by deletion of at least two capsid genes, with appropriate helper, can be used in P3 conditions to propagate DNA sequences from:

III-C-1-c-(1)-(a)-(*1*). Bacteria of Class 1 or Class 2[1] or their phages or plasmids except for those that produce potent polypeptide toxins; (*34*)

III-C-1-c-(1)-(a)-(2). Eukaryotic organisms that do not produce potent polypeptide toxins(*34*) (shotgun experiments or purified DNA).

III-C-1-c-(1)-(b). Experiments involving the use of unconditionally defective human adenovirus 2 and 5 genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by NIH on a case-by-case basis(*45*) and will be conducted under the recommended physical containment conditions.

III-C-1-c-(2). *Non-productive virus-cell interactions.* Defective or intact human adenovirus 2 and 5 genomes can be used as vectors in P2 conditions to transform nonpermissive cells in culture, provided the inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis.(*45*)

III-C-1-d. *Murine Adenovirus Strain FL.*

III-C-1-d-(1). *Productive Virus-Cell Interactions.*

III-C-1-d-(1)-(a). Unconditionally defective murine adenovirus strain FL genomes, with appropriate helper, can be used in P2 conditions to propagate DNA sequences from:

III-C-1-d-(1)-(a)-(*1*). Bacteria of Class 1 or Class 2 [1] or their phages or plasmids except for those that produce potent polypeptide toxins;(*34*)

III-C-1-d-(1)-(a)-(*2*). Eukaryotic organisms that do not produce potent polypeptide toxins(*34*) (shotgun experiments or purified DNA).

III-C-1-d-(1)-(b). Experiments involving the use of intact murine adenovirus strain FL genomes to propagate DNA sequences from prokaryotic or eukaryotic organisms will be evaluated by NIH on a case-by-case basis(*45*) and will be conducted under the recommended physical containment conditions.

III-C-1-d-(1)-(c). Experiments involving the use of unconditionally defective murine adenovirus strain FL genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by NIH on a case-by-case basis(*45*) and will be conducted under the recommended physical containment conditions.

III-C-1-d-(2). *Non-Productive Virus-Cell Interactions.* Defective or intact murine adenovirus strain FL genomes can be used as vectors in P2 conditions to transform nonpermissive cells in culture, provided the inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis(*45*).

III-C-1-e. *All Other Potential Viral Vectors.*

III-C-1-e-(1). Experiments involving recombinant DNA molecules containing viral DNA segments consisting of 25 percent or less of the virus genome can be done:

III-C-1-e(1)-(a). In P2 conditions when the recombinant DNA is to be integrated into the cell genome or is known to replicate as a plasmid in cells in culture, provided the additional DNA sequences are not derived from a eukaryotic virus. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis;(*45*).

III-C-1-e—(1)-(b). Under physical containment conditions to be determined by NIH(*45*) when a viral helper will be used to propagate DNA sequences from prokaryotic or eukaryotic organisms.

III-C-1-e-(2). Experiments involving the use of other intact or defective virus genomes to propagate DNA sequences from prokaryotic or eukaryotic organisms (and viruses), or as vectors to transform nonpermissive cells, will be evaluated by NIH on a case-by-case basis(*45*) and will be conducted under the recommended physical containment conditions.

NIH will also review all experiments involving the use of virus vectors in animals and the physical containment conditions appropriate for such studies.

III-C-2. *Invertebrate Host-Vector Systems in Which Insect Viruses Are Used To Propagate Other DNA Segments.* As soon as information becomes available on the host range restrictions and on the infectivity, persistance, and integration of the viral DNA in vertebrate and invertebrate cells, experiments involving the use of insect viruses to propagate DNA sequences will be evaluated by NIH on a case-by-case basis(*45*) and will be conducted under the recommended physical containment conditions. Experiments should be done in established invertebrate cell lines and should follow, where appropriate, criteria recommended for vertebrate viral DNA vectors (see Section III-C-1).

III-C-3. *Plant Viral Host-Vector Systems.* The DNA plan viruses which could currently serve as vectors for cloning genes in plants and plant cell protoplasts are Cauliflower Mosaic Virus (CaMV) and its close relatives, which have relaxed circular double-stranded DNA genomes with a molecular weight of 4.5×10^6, and Bean Golden Mosaic Virus (BGMV) and re-

lated viruses with small ($<10^6$ daltons) single-stranded DNA genomes. These viruses are not known to integrate into host chromosomes or to incorporate cellular genes into their genomes. CaMV is spread in nature by aphids, in which it survives for a few hours. Spontaneous mutants of CaMV which lack a factor essential for aphid transmission arise frequently. BGMV is spread in nature by whiteflies, and certain other single-stranded DNA plant viruses are transmitted by leafhoppers. These single-stranded plant viruses persist for days or weeks in their insect vectors, but are thought not to replicate there.

The DNA plant viruses have narrow host ranges and are relatively difficult to transmit mechanically to plants. For this reason, they are most unlikely to be accidentally transmitted from spillage of purified virus preparations.

When these viruses are used as vectors in intact plants, or propagative plant parts, the plants should be grown under P1 conditions—that is, in either a limited access greenhouse or plant growth cabinet which is insect-proof, preferably with positive air pressure, and in which an insect fumigation regime is maintained. Soil, plant pots, and unwanted infected plant materials should be removed from the greenhouse or cabinet in sealed insect-proof containers and sterilized. It is not necessary to sterilize run-off water from the infected plants, as this is not a plausible route for secondary infection. When the viruses are used as vectors in tissue cultures or in small plants in axenic cultures, no special containment is recommended. Infected plant materials which have to be removed from the greenhouse or cabinet for further research, should be maintained under insect-proof conditions. These measures provide an entirely adequate degree of containment. They are similar to those required in many countries for licensed handling of "exotic" plant viruses.

The CaMV strain used as a cloning vector should be a mutant that lacks the aphid transmission factor.

The viruses or their DNA may also be useful as vectors to introduce genes into plant protoplasts. The fragility of plant protoplasts combined with the properties of the viruses mentioned above provide adequate safety. Since no risk to the environment from the use of the DNA plant virus/protoplast system is envisaged, no special containment is recommended, except as described in the following paragraph.

Experiments involving the use of plant virus genomes to propagate DNA sequences from eukaryotic viruses will be evaluated on a case-by-case basis(*45*) and will be conducted under the recommended containment conditions.

Table IV

Recommended Containment for Recombinant DNA Research Using Eukaryotic Viral Vectors
(See text for full details)

Vector DNA	Productive virus-cell interactions						Nonproductive virus-cell interactions[46]
	Type of DNA insert					Eukaryotic viral	
	Prokaryotic		Eukaryotic				
	Shotgun	Purified	Shotgun		Purified[47]		
			Natural host	Other			
1. Polyoma							
Intact Genome	P2	P2	P2	P3	P2	CBC*	P2
Deleted Genome	P2	P2	P2	P2	P2	CBC*	P2
2. SV40							
Intact Genome	—	—	—	—	—	—	P2
Deleted Genome	P2	P2	P2	P3	P3	CBC*	P2
3. Human Ad2+Ad5							
Deleted Genome	P3	P3	P3	P3	P3	CBC*	P2
4. Mouse Adenovirus (Strain FL)							
Intact Genome	CBC*	CBC*	CBC*	CBC*	CBC*	CBC*	P2
Deleted Genome	P2	P2	P2	P2	P2	CBC*	P2
5. Insect Viruses	CBC*	CBC*	CBC*	CBC*	CBC*	CBC*	—
6. Plant Viruses (CaMV and BGMV)	**	**	**	**	**	CBC*	—
7. All other potential Viral Vectors	CBC*	CBC*	CBC*	CBC*	CBC*	CBC*	CBC*

*CBC - Case-by-case[45]
**See text

III-C-4. *Plant Host-Vector Systems Other than Viruses.* Organelle, plasmid, and chromosomal DNA's may be used as vectors. DNA recombinants formed between such vectors and host DNA, when propagated only in that host (or a closely related strain of the same species), are exempt from these Guidelines (see section I-E). DNA recombinants formed between such vectors and DNA from cells other than the host species require P2 physical containment. The development and use of host-vector systems that exhibit a high level of biological containment, such as those using protoplasts or undifferentiated cells in culture, permit a decrease in the physical containment to P1.

Intact plants or propagative plant parts which because of their large size cannot be grown in a standard P2 laboratory may be grown under the P1 conditions described above in section III-C-3 except that (i) sterilization of run-off water is required where this is a plausible route for secondary infection and (ii) the standard P2 practices are adopted for microbiological work.

III-C-5. *Fungal or Similar Lower Eukaryotic Host-Vector Systems.* The containment criteria for DNA recombinant experiments using these host-vectors most closely resemble those for prokaryotes, rather than those for the preceding eukaryotes, since the host cells usually exhibit a capacity for dissemination outside the laboratory that is similar to that for bacteria. Therefore, the procedures established for certification of HV systems other than *E. coli* K-12 (sec. II-D-2) will also apply to these fungal or similar lower eukaryotic host-vector systems.

Once an HV1 system is approved by NIH, it may be used under P2 containment for shotgun experiments with phages, plasmids, and DNA from Class 1 prokaryotes(*1*) and lower eukaryotes that do not produce polypeptide toxins.(*34*) Other classes of recombinant DNA experiments with these HV1 systems will require prior approval and classification by NIH. Should HV2 or HV3 systems of this type be developed and approved by NIH, guidelines for their use in other types of recombinant DNA experiments will also be established.

In addition to the experiments described above, the following experiments maybe carried out without the eukaryotic host (Host C) having been approved as an HV1 host: DNA from Host C may be inserted into a vector from a certified EK2 host-vector system and propagated in *E. coli* K-12 under the appropriate containment conditions [see Section III-A-1-(a)-(5)]. Subsequently, this recombinant DNA may be returned to Host C and propagated there under P1 conditions. (*43*)

III-D. *Complementary DNA's.* Specific containment levels are given in Section III-A-2-a (see also last column of Table III) for complementary DNA (cDNA) of viral mRNA. For the other Sections of the Guidelines, where applicable, cDNA's synthesized *in vitro* are included within each of the above classifications. For example, cDNA's formed from cellular RNA's that are not purified and characterized are included under III-A-1, shotgun experiments; cDNA's formed from purified and characterized RNA's are included under III-A-3; etc.

Due to the possibility of nucleic acid contamination of enzyme preparations used in the preparation of cDNA's, the investigator must employ purified enzyme preparations that are free of viral nucleic acid.

III-E *Synthetic DNA's.* If the synthetic DNA segment could yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent), the containment conditions must be as stringent as would be used for propagating the natural DNA counterpart.

If the synthetic DNA sequence codes for a harmless product, it may be propagated at the same containment level as its purified natural DNA counterpart. For example, a synthetic DNA segment, to be propagated in *E. coli* K-12, which corresponds to a nonharmful gene of birds, would require P2 physical containment plus an EK1 host-vector, or P1 + EK2.

If the synthetic DNA segment is not expressed *in vivo* as a polynucleotide or polypeptide product, the organisms containing the recombinant DNA molecules are exempt(*4*) from the Guidelines.

IV. Roles and Responsibilities

Safety involving recombinant DNA molecules depends primarily on the individuals conducting the research activities. The guidelines cannot anticipate every possible situation. Motivation and good judgment are the keys to protection of health and the environment.

The guidelines are designed to help the principal investigator determine the safeguards that should be implemented. They will never be complete and final, since all conceivable experiments involving recombinant DNA cannot be foreseen. Therefore, it is the principal investigator's responsibility to insure that the purpose of the guidelines is fulfilled.

The institution, and the Institutional Biosafety Committee (IBC) acting on its behalf, are given responsibility for seeing that recombinant DNA activities comply with the guidelines. This delegation of authority will serve the scientific process and at the same time properly focus accountability for safe conduct of the research.

The following roles and responsibilities constitute an administrative framework in which safety is an essential and integral part of research involving recombinant DNA molecules. Detailed administrative procedures designed to implement this framework are provided in Appendix C. Further clarifications and interpretations of roles and responsibilities will be issued by NIH as necessary.

IV-A. *Responsibilities of the Institution*

IV-A-1. *Institution.* The institution bears primary responsibility for establishing and implementing policies for the safe conduct of research involving recombinant DNA molecules. These shall be policies that assure compliance with the NIH Guidelines. In carrying out its responsibilities, the institution shall:

IV-A-1-a. establish an Institutional Biosafety Committee (IBC) and insure that it is fulfilling its responsibilities;

IV-A-1-b. report to the NIH Office of Recombinant DNA Activities (ORDA) the names of members of its IBC and relevant information on them;

IV-A-1-c. submit to NIH for registration a Memorandum of Understanding and Agreement (MUA) or equivalent information (in the case of non/NIH supported recombinant DNA projects), approved by the IBC, for all recombinant DNA research at an institution receiving any NIH funds for recombinant DNA research (see section IV-C and Appendix C for NIH policies on MUAs and other required documentation);

IV-A-1-d. Assume responsibility for insuring compliance of recombinant DNA projects with the procedures and standards of the NIH Guidelines. If, upon registration and review, NIH (ORDA) finds that IBC approved protocols do not conform with standards set forth in the NIH Guidelines, the institution will be notified by NIH and shall take appropriate action to bring the protocols into compliance (see Appendix C for additional information). Further, the institution shall insure that all principal investigators, irrespective of source of funding, have agreed to carry out their responsibilities under the Guidelines;

IV-A-1-e. Determine, in connection with each project, the necessity for medical surveillance of recombinant DNA research personnel before, during, and after their involvment in this research. Where possible, each institution should cooperate with the local public health department. An institution's medical surveillance program might include, for example, records of agents handled, active investi-

gation of relevant illnesses acquired by recombinant DNA research personnel, and the maintenance of serial serum samples (see "Laboratory Safety Monograph—A Supplement to the NIH Guidelines for Recombinant DNA Research" for additional information on medical surveillance);

IV-A-1-f. Establish rules as necessary to implement the Guidelines.

IV-A-2. *Institutional Biosafety Committee.* The principal functions of the IBC are to review and oversee all recombinant DNA projects and to advise the institution and ORDA whether the proposals and the research comply with the NIH Guidelines.

The IBC shall be a committee of not less than five members so selected that the committee has the experience and expertise to assess the safety of proposed recombinant DNA research projects and any potential risks to public health or the environment. Its membership should include individuals from disciplines relevant to recombinant DNA technology, biological safety, and engineering. It is recommended that the IBC also include members knowledgeable about such matters as applicable law, standards of professional conduct and practice, community attitudes, and the environment. It is recommended that at least one member be a nondoctoral person from a laboratory technical staff. No member of an IBC may be involved (except to provide information requested by the IBC) in the review or approval of a project in which he or she has been, or excepts to be engaged, or has a direct finanical interest. At least one member shall not be affiliated with the institution, i.e., no IBC may consist entirely of persons who are officers, employees, or agents of the institution, or are otherwise associated with it apart from their membership on the IBC.

On behalf of the institution, the IBC shall:

IV-A-2-a. Review and, if in compliance with the NIH Guidelines, approved the initiation of all proposed recombinant DNA research conducted at or sponsored by the institution receiving NIH funds for any recombinant DNA research. (All P4 research must recieve prior approval by NIH before its initiation.) This review shall include (i) an independent assessment of the containment levels required by these guidelines for the proposed research, and (ii) review and approval of facilities, procedures, practices, and the training and expertise of recombinant DNA personnel;

IV-A-2-b. Consider requests for approval of single-step reductions in containment levels for experiments with purified DNA and characterized clones and report to ORDA those actions in which approval is given (see section III-A-3);

IV-A-2-c. Review periodically recombinant DNA research being conducted at the institutions;

IV-A-2-d. Review and approve emergency plans covering accidential spills and personnel contamination resulting from this research;

IV-A-2-e. Report promptly to ORDA any problems with the Guidelines of violations;

IV-A-2-f. Keep minutes of meetings and, upon request, make them available to the public;

IV-A-2-g. Otherwise advise the institution and ORDA on policy matters relating to recombinant DNA research.

Appendix C and "Laboratory Safety Monagraph—A Supplement to the NIH Guidelines for Recombinant DNA Research" contain additional information and suggestions regarding the function and operation of IBCs.

IV-A-3. *Biological Safety Officer.* A biological safety officer shall be designated by each institution engaged in recombinant DNA research at the P3 or P4 containment level. The officer shall be a member of the IBC. His or her duties shall include, but need not be limited to:

IV-A-3-a. Insuring through periodic inspections that laboratory safety standards are rigorously followed;

IV-A-3-b. Developing emergency plans for dealing with accidental spills and personnel contamination, and investigating recombinant DNA research laboratory accidents;

IV-A-3-c. Providing advice on laboratory security;

IV-A-3-d. Providing technical advice to the principal investigator and IBC on research safety procedures.

See "Laboratory Safety Monograph—A Supplement to the NIH Guidelines for Recombinant DNA Research" for additional information on the duties of the Biological Safety Officer.

IV-A-4. *Principal Investigator.* The principal investigator is responsible for conducting his or her recombinant DNA research in compliance with the NIH Guidelines. On behalf of the institiution, the principal investigators responsible for:

IV-A-4-a. Complying fully with the guidelines in carrying out the research;

IV-A-4-b. Making the initial determination of the required levels of physical and biological containment in accordance with the guidelines;

IV-A-4-c. Selecting appropriate microbiological practices and laboratory techniques to be used in the research;

IV-A-4-d. Being adequately trained in good microbiological techniques;

IV-A-4-e. Submitting the proposed research (including subsequent changes in the protocol—e.g., changes in the source of DNA or host-vector system to be used) to the IBC for review and approval or disapproval, and remaining in communication with the IBC throughout the conduct of the project;

IV-A-4-f. Initiating no recombinant DNA research subject to the guidelines until it has been approved by the IBC and has met all other requirements of the guidelines, and agreeing to make changes to conform if ORDA's review so requires;

IV-A-4-g. Petitioning NIH, after notifying the IBC, for exceptions or exemptions (4) to these guidelines—e.g., for an exception to a prohibition (see section I-D) or an exemption from the guidelines (see section I-E-4 and I-E-5). Each request for such an exception or exemption must be accompanied by adequate documentation (see appendix C for additional information on procedures);

IV-A-4-h. Reporting promptly to the IBC and NIH (ORDA) any problems with or violations of the guidelines;

IV-A-4-i. Submitting information on proposed new host-vector systems to ORDA in order to have them certified;

IV-A-4-j. Reporting to the IBC and ORDA new information bearing on the guidelines;

IV-A-4-k. Adhering to IBC-approved emergency plans for dealing with accidental spills and personnel contamination;

IV-A-4-l. Complying with shipping requirements for recombinant DNA molecules (see section II-C, appendix C, and "Laboratory Safety Monograph—A Supplement to the NIH Guidelines for Recombinant DNA Research" for instructions on shipping and distributing organisms and viruses containing recombinant DNA);

IV-A-4-m. After receiving IBC approval and before initiating the research, the principal investigator is responsible for:

IV-A-4-m-(1). Making available to the laboratory staff copies of the approved protocols that describe the potential biohazards and the precautions to be taken;

IV-A-4-m-(2). Instructing and training the staff in the practices and techniques required to insure safety and in the procedures for dealing with accidents;

IV-A-4-m-(3). Informing the staff of the reasons and provisions for any advised or requested precautionary medical practices, such as vaccinations or serum collection.

IV-A-4-n. During the conduct of the research, the principal investigator is responsible for:

IV-A-4-n-(1). Supervising the safety performance of the staff to insure

that the required safety practices and techniques are employed;

IV-A-4-n-(2). Investigating and reporting in writing to ORDA and the IBC any serious or extended illnesses of a worker or any accident that results in (i) inoculation of recombinant DNA materials through cutaneous penetration, (ii) ingestion of recombinant DNA materials, (iii) probable inhalation of recombinant DNA materials following gross aerosolization, or (iv) any incident causing serious exposure to personnel or danger of environmental contamination;

IV-A-4-n-(3). Investigating and reporting in writing to ORDA, the biological safety officer (where applicable), and the IBC any significant problems pertaining to operation and implementation of biological and physical containment practices and procedures;

IV-A-4-n-(4). Correcting work errors and conditions that may result in the release of recombinant DNA materials;

IV-A-4-n-(5). Insuring the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., genotypic and phenotypic characteristics, purity, etc.).

See "Laboratory Safety Monograph—A Supplement to NIH Guidelines for Recombinant DNA Research" for additional information on training and laboratory and accident procedures.

IV-A-4-o. While not a requirement, it is urged that all publications dealing with recombinant DNA research include a description of the physical and biological containment procedures employed, to aid others who might consider repeating the work.

IV-B. *Responsibilities of NIH.*

IV-B-1. *Office of the Director, NIH.* The Office of the Director shall be responsible for:

IV-B-1-a. Final interpretation of the guidelines;

IV-B-1-b. Revision and amendment of the guidelines after appropriate notice and opportunity for public comment;

IV-B-1-c. Certification of new host-vector systems and decertification of existing host-vector systems after appropriate notice and opportunity for public comment (see Section II-D-2-a);

IV-B-1d. Promulgating and amending a list of classes of recombinant DNA molecules to be exempt(*4*) from these guidelines after appropriate notice and opportunity for public comment, if it is found that they consist entirely of DNA segments from different species that exchange DNA by known physiological processes or otherwise do not present a significant risk to health or environment (see section I-E-4 and I-E-5);

IV-B-1-e. Permitting, after appropriate notice and opportunity for public comment, exceptions(*4*) to the Prohibitions in the guidelines for experiments—e.g., risk-assessment studies. In making such decisions on exceptions, weight will be given both to scientific and societal benefits and to potential risks (see section I-D). Also, approving changes in containment levels for specific experiments, or the assignment of levels to experiments not explicitly considered in the guidelines (see part III). Also, designating as class 1 for purposes of these guidelines certain agents which are listed as class 2 (see footnote 1);

IV-B-1-f. Overseeing the implementation of the guidelines;

IV-B-1-g. Requesting, when appropriate, the advice of the Advisory Committee to the Director, NIH, on matters relevant to recombinant DNA policy issues;

IV-B-1-h. Promulgating rules as necessary to implement the guidelines.

IV-B-2. *NIH Recombinant DNA Advisory Committee.* The duties of the Recombinant DNA Advisory Committee (RAC) shall include:

IV-B-2-a. Recommending to the Director, NIH, revisions of these guidelines periodically and any amendments to the guidelines as necessary;

IV-B-2-b. Advising the Director, NIH, and ORDA on questions of interpretation of the guidelines;

IV-B-2-c. Recommending to the Director, NIH whether host-vector systems qualify for certification (see section II-D-2-a);

IV-B-2-d. Recommending to the Director, NIH, whether currently certified host-vector systems should be decertified;

IV-B-2-e. Recommending to the Director, NIH, a list (and amendments to the list) of other classes of experiments to be exempt(*4*) from these guidelines (see section I-E-4 and I-E-5);

IV-B-2-f. Recommending to the Director, NIH, whether experiments should be granted an exception (*4*) from the prohibitions in the guidelines—for example, in order to allow risk-assessment studies—and at the same time recommending appropriate levels of physical and biological containment for these experiments. In making such recommendations, weight shall be given both to scientific and societal benefits and to potential risks (see section I-D);

IV-B-2-g. Recommending to the Director, NIH, changes in containment levels for specific experiments, or the assignment of levels to experiments not explicitly considered in the guidelines (see part III);

IV-B-2-h. Recommending to the Director, NIH, designation as class 1 for purposes of these guidelines certain agents that are listed as class 2 (see footnote 1);

IV-B-2-i. Recommending to NIH whether a cloned recombinant DNA segment has been rigorously characterized and whether there is sufficient evidence that it is free of harmful genes, so that experiments involving it may be conducted under lower containment conditions;

IV-B-2-j. Carrying out other functions as assigned under the RAC's charter or by the Secretary, the Assistant Secretary for Health, or the Director, NIH.

IV-B-3. *NIH Components.* Various NIH components shall perform the following:

IV-B-3-a. *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, biosafety committees, principal investigators, and State and local governments. In addition, ORDA shall:

IV-B-3-a-(1). Make an independent evaluation of the containment levels required for the research covered by these guidelines;

IV-B-3-a-(2). Determine whether the physical and biological containment levels approved by the IBC are in accord with the requirements of the guidelines;

IV-B-3-a-(3). Make interpertations of the guidelines and approve reduction of containment levels of more than one step for characterized clones, or for cases involving primate DNA, or to levels below P1+EK1 (see section III-A-3). In most cases this will involve prior review by the RAC;

IV-B-3-a-(4). Provide timely notice to local institutions when protocols, including modifications to ongoing projects, do not conform to the standards in the NIH guidelines;

IV-B-3-a-(5). Maintain a register of recombinant DNA projects;

IV-B-3-a-(6). Serve as executive secretariat for the RAC;

IV-B-3-a-(7). Publish the *Recombinant DNA Technical Bulletin;*

IV-B-3-a-(8). Review membership of IBC's.

IV-B-3-b. *Other NIH Components.* Other NIH components shall be responsible for:

IV-B-3-b-(1). Awarding no grants or contracts unless properly executed MUAs have been received;

IV-B-3-b-(2). Certifying P4 facilities, inspecting them periodically, and inspecting other recombinant DNA facilities as deemed necessary;

IV-B-3-b-(3). Announcing and distributing certified HV2 and HV3 host-vector systems (see section II-D-3).

See Appendix C for additional information on the administrative procedures of ORDA and other NIH components.

IV-C. *Registration.*

IV-C-1. *Required Registration.* All institutions receiving NIH funds for recombinant DNA projects shall inform NIH of all recombinant DNA projects at the institution. A non-NIH project shall be registered with NIH after it has been approved by the IBC and initiated. Applications for NIH projects must be accompanied by an MUA.

For information on MUA's or equivalent documents, which must be submitted for registration of recombinant DNA projects, see section IV of appendix C.

IV-C-2. *Voluntary Registration and Certification.* Any institution which is not required to comply with the guidelines may nevertheless register recombinant DNA research projects with NIH by submitting the appropriate information to ORDA. NIH will accept requests for certification of host-vector systems proposed by the institution. The submitter must agree to abide by the physical and biological containment standards of the NIH guidelines.

IV-C-3. *Disclosure of Information*

IV-C-3-a. DHEW or the institution, in carrying out their responsibilities under the guidelines, shall not release confidential or proprietary information submitted pursuant to the guidelines, except to the extent:

IV-C-3-a-(1). Required by law;

IV-C-3-a-(2). Necessary to certify host-Vector systems;

IV-C-3-a-(3). Necessary to determine whether or not to allow exemptions from the guidelines;

IV-C-3-a-(4). Necessary, in the judgment of the Secretary or his designee, the protect the public or the environment against an unreasonable risk of injury to health or the environment.

IV-C-4. *Patentable Material.* Institutions are reminded that whenever they regard information as potentially proprietary, they should consider applying for a patent before submitting information to DHEW.

IV-D. *Compliance.* As a condition for NIH funding of recombinant DNA research, institutions must insure that recombinant DNA research conducted at or sponsored by that institution shall comply with the guidelines irrespective of the source of funding.

IV-D-1. *Policy on Noncompliance*

IV-D-1-a. All NIH-funded projects involving recombinant DNA technology must comply with the NIH guidelines. Noncompliance may result in suspension, limitation, or termination of financial assistance for such projects, and for other recombinant DNA research at the institution.

IV-D-1-b. All non-NIH funded projects involving recombinant DNA techniques conducted at or sponsored by an institution that receives NIH funds for projects involving recombinant DNA techniques must comply with the NIH guidelines. Noncompliance may result in suspension, limitation, or termination of NIH funds for recombinant DNA research.

IV-D-1-c. Information concerning noncompliance with the guidelines may be brought forward by any person. It should be delivered to both ORDA and the relevant institution. The institution, generally through the IBC, shall take such action as appropriate. It shall forward a complete report of the incident to ORDA and, if appropriate, shall include recommendations for further action.

IV-D-1-d. In cases where NIH proposes to suspend, limit, or terminate financial assistance because of a noncompliance with the guidelines, applicable HEW and PHS procedures shall govern. Volume 42, parts 50 and 52, and volume 45, parts 16 and 74, of the Code of Federal Regulations are sources of information about these procedures for grants.

V. Footnotes And References

1. The reference to organisms as class 1, 2, 3, 4, or 5 refers to the classification in the publication *Classification of Etiologic Agents on the Basis of Hazard*, 4th edition, July 1974; U.S. Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, Office of Biosafety, Atlanta, Ga. 30333. The list of organisms in each class, as given in this publication, is reprinted in appendix B to these guidelines.

However, the Director, NIH, on the recommendation of the Recombinant DNA Advisory Committee, may designate certain of the agents which are listed as class 2 in the *Classification of Etiologic Agents on the Basis of Hazard*, 4th edition, July 1974, as class 1 agents for the purposes of these guidelines. An updated list of such agents may be obtained from the Office of Recombinant DNA Activities (ORDA), National Institutes of Health, Bethesda, Md. 20014.

The entire *Classification of Etiologic Agents on the Basis of Hazard* is in the process of revision.

2. One exception to the prohibition of formation of recombinant DNA's derived from class 3, 4, or 5 agents is that the formation of recombinant DNA's derived from Vesicular Stomatitis Virus (VSV) is not prohibited. The reason for this is explained in the accompanying "Decision Document." However, as noted in appendix B, a permit form the U.S. Department of Agriculture is required for the import or interstate transport of VSV. This can be obtained from USDA-APHIS, Veterinary Service, Federal Building, Hyattsville, Md. 20782.

3. The following types of data should be considered in determining whether DNA recombinants are "characterized" and "free of harmful genes": (a) the absence of potentially harmful genes (e.g., sequences contained in indigenous tumor viruses or sequences that code for toxins, invasins, virulence factors, etc., that might potentiate the pathogenicity or communicability of the vector and/or host or be detrimental to humans, animals, or plants); (b) the types(s) of genetic information on the cloned segment and the nature of transcriptional and translation gene products specified; (c) the relationship between the recovered and desired segment (e.g., hybridization and restriction endonuclease fragmentation analysis where applicable); (d) the genetic stability of the cloned fragment; and (e), any alterations in the biological properties of the vector and host.

4. In section I-E, " exemptions" from the guidelines are discussed. Such experiments are not covered by the guidelines and need not be registered with NIH. In section I-D on "prohibitions," the possibility of "exceptions" is discussed. An "exception" means that an experiment may be expressly released from a prohibition. At that time it will be assigned appropriate levels of physical and biological containment.

5. See "Laboratory Safety Monograph—A Supplement to the NIH Guidelines for Recombinant DNA Research" for information on inactivating DNA.

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

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

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

9. *National Institutes of Health Biohazards Safety Guide* (1974). U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health. U.S. Government Printing Office, Stock No. 1740-00383.

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

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

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

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20. Biological safety cabinets referred to in this section are classified as *class I, class II,* or *class III* cabinets. A *class I* is a ventilated cabinet for personnel protection having an inward flow of air away from the operator. The exhaust air from this cabinet is filtered through a high-efficiency particulate air (HEPA) filter. This cabinet is used in three operational modes: (1) with a full-width open front, (2) with an installed front closure panel (having four 8-inch diameter openings) without gloves, and (3) with an installed front closure panel equipped with arm-length rubber gloves. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater. A *class II* cabinet is a ventilated cabinet for personnel and product protection having an open front with inward air flow for personnel protection, and HEPA filtered mass recirculated air flow for product protection. The cabinet exhaust air is filtered through a HEPA filter. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater. Design and performance specifications for *class II* cabinets have been adopted by the National Sanitation Foundation, Ann Arbor, Mich. A *class III* cabinet is a closed-front ventilated cabinet of gas-tight construction which provides the highest level of personnel protection of all biohazard safety cabinets. The interior of the cabinet is protected from contaminants exterior to the cabinet. The cabinet is fitted with arm-length rubber gloves and is operated under a negative pressure of at least 0.5 inch water gauge. All supply air is filtered through HEPA filters. Exhaust air is filtered through two HEPA filters or one HEPA filter and incinerator before being discharged to the outside environment.

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34. We are specifically concerned with potent toxins which could be produced by acquiring a single gene or cluster of genes.

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

36. As classified in the Second Report of the International Committee on Taxonomy of Viruses: Classification and Nomenclature of Viruses, Frank Fenner, Ed. Intervirology 7 (19-115) 1976. (As noted in the Prohibition Section, the use of viruses classified[1] as class 3, 4, or 5, other than VSV, is prohibited.)

37. The cDNA copy of the viral mRNA must be >99 percent pure; otherwise as for shotgun experiments with eukaryotic cellular DNA.

38. >99 percent pure (i.e., less than 1 percent of the DNA consists of intact viral genames); otherwise as for whole genames.

39. The viruses have been classified by NCI as "moderate risk oncogenic viruses." See "Laboratory Safety Monograph—A Supplement to the NIH Guidelines for Recombinant DNA Research" for recomendations on handling the viruses themselves.

40. EKICV means the use of an EK1 host and a vector certified for use in an EK2 system.

41. The DNA preparation is defined as "purified" if the desired DNA represents at least 99 percent (w/w) of the total DNA in the preparation, provided that it was verified by more than one procedure.

42. The lowering of the containment level when this degree of purification has been obtained is based on the fact that the total number of clones that must be examined to obtain the desired clone is markedly reduced. Thus, the probability of cloning a harmful gene could, for example, be reduced by more than 10^3-fold when a nonrepetitive gene from mammals was being sought. Furthermore, the level of purity specified here makes it easier to establish that the desired DNA does not contain harmful genes.

43. This is not permitted, of course, if it falls under any of the Prohibitions of section I-D. Of particular concern here is prohibition (v), i.e., "Transfer of a drug resistance trait to microorganisms that are not known to acquire it naturally if such acquisition could compromise the use of a drug to control disease agents in human or veterinary medicine or agriculture."

44. Because this work will be done almost exclusively in tissue culture cells, which have no capacity for propagation outside the laboratory, the primary focus for containment is the vector. It should be pointed out that risk of laboratory-acquired infection as a consequence of tissue culture manipulation is very low. Given good microbiological practices, the most likely mode of escape of recombinant DNA's from a physically contained laboratory is carriage by an infected human. Thus the vector with an inserted DNA segment should have little or no ability to replicate or spread in humans.

For use as a vector in a vertebrate host cell system, an animal viral DNA molecule should display the following properties:

(i) It should not consist of the whole genome of any agent that is infectious for humans or that replicates to a significant extent in human cells in tissue culture. If the recombinant molecule is used to transform nonpermissive cells (i.e., cells which do not produce infectious virus particles), this is not a requirement.

(ii) It should be derived from a virus whose epidemiological behavior and host range are well understood.

(iii) In permissive cells, it should be defective when carrying an inserted DNA segment (i.e., propagation of the recombinant DNA as a virus must be dependent upon the presence of a complementing helper genome). In almost all cases this condition would be achieved automatically by the manipulations used to construct and propagate the recombinants. In addition, the amount of DNA encapsidated in the particles of most animal viruses is defined within fairly close limits. The insertion of sizable foreign DNA sequences, therefore, generally demands a compensatory deletion of viral sequences. It may be possible to introduce very short insertions (50-100 base pairs) without rendering the viral vector defective. In such a situation, the requirement that the viral vector be defective is not necessary, except in those cases in which the inserted DNA encodes a bilogically active polypeptide.

It is desired but not required that the functional anatomy of the vector be known—that is, there should be a clear idea of the location within the molecule of:

(i) the sited at which DNA synthesis originates and terminates,

(ii) the sites that are cleaved by restriction endonucleases,

(iii) the template regions for the major gene product.

If possible the helper virus genome should:

(i) be integrated into the genome of a stable line of host cells (a situation that would effectively limit the growth of the vector recombinant to such cell lines) or

(ii) consist of a defective genome, or an appropriate conditional lethal mutant virus, making vector and helper dependent upon each other for propagation.

However, neither of these stipulations is a requirement.

45. Review by NIH on a case-by-case basis means prior review and the setting or appropriate containment conditions by NIH. NIH actions in such case-by-case reviews will be published in the *Recombinant DNA Technical Bulletin.*

46. Provided the inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated on a case-by-case basis.

47. ≥ 99% pure; otherwise as for shotgun experiments.

APPENDIX A

Section I-E-4 states that exempt from these Guidelines are "certain specified recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such exchanges will be prepared and periodically revised by the Director, NIH, on the recommendation of the Recombinant DNA Advisory Committee, after appropriate notice and opportunity for public comment. Certain classes are exempt as of publication of these Revised Guidelines. The list is in Appendix A."

Under exemption I-E-4, as of the publication of these Revised Guidelines, are any recombinant DNA molecules that are (1) composed entirely of DNA segments from one or more of the organisms listed below and (2) to be propagated in any of the organisms listed below.

Escherichia species
Edwardsiella species
Citrobacter species (including *Levinea*)
Salmonella species (including *Arizona*)
Shigella species
Klebsiella species
Enterobacter species
Hafnia species
Serratia species
Erwinia species (including *Pectobacterium*)
Pseudomonas species
Rhizobium species
Acinetobacter calcoaceticus
Agrobacterium tumefaciens
Rhodopseudomonas sphaeroides

APPENDIX B

CLASSIFICATION OF MICROORGANISMS ON THE BASIS OF HAZARD*

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

A. CLASS 1 AGENTS

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

B. CLASS 2 AGENTS

1. Bacterial agents:
Actinobacillus—all species except *A. mallei*, which is in Class 3
Arizona hinshawii—all serotypes
Bacillus anthracis
Bordetella—all species
Borrelia recurrentis, B. vincenti
Clostridium botulinum, Cl. chauvoei, Cl. haemolyticum, Cl. histolyticum, Cl. novyi, Cl. septicum, Cl. tetani
Corynebacterium diphtheriae, C. equi, C. haemolyticum, C. pseudotuberculosis, C. pyogenes, C. renale
Diplococcus (Streptococcus) pneumoniae
Erysipelothrix insidiosa
Escherichia coli—all enteropathogenic serotypes
Haemophilus ducreyi, H. influenzae
Herellea vaginicola
Klebsiella—all species and all serotypes
Leptospira interrogans—all serotypes
Listeria—all species
Mima polymorpha
Moraxella—all species
Mycobacteria—all species except those listed in Class 3
Mycoplasma—all species except *Mycoplasma mycoides* and *Mycoplasma agalactiae*, which are in Class 5
Neisseria gonorrhoeae, N. meningitidis
Pasteurella—all species except those listed in Class 3
Salmonella—all species and all serotypes
Shigella—all species and all serotypes
Sphaerophorus necrophorus
Staphylococcus aureus
Streptobacilus moniliformis
Streptococcus pyogenes
Treponema carateum, T. pallidum, and *T. pertenue*
Bibrio fetus, V. comma, including biotype El Tor, and *V. parahemolyticus*
2. Fungal agents:
Actinomycetes (including *Nocardia* species and *Actinomyces* species and *Arachnia propionica*)
Blastomyces dermatitidis
Cryptococcus neoformans
Paracoccidioides brasiliensis
3. Parasitic agents:
Endamoeba histolytica
Leishmania sp.
Naegleria gruberi
Toxoplasma gondii
Toxocara canis
Trichinella spiralis
Trypanosoma cruzi
4. Viral, Rickettsial, and Chlamydial agents:
Adenoviruses—human—all types
Cache Valley virus
Coxsackie A and *B viruses*
Cytomegaloviruses
Echoviruses—all types
Encephalomyocarditis virus (EMC)
Flanders virus
Hart Park virus
Hepatitis-associated antigen material
Herpes viruses—except *Herpesvirus simiae* (Monkey B virus) which is in Class 4
Corona virus
Influenza viruses—all types except A/PR8/34, which is in Class 1
Langet virus
Lymphogranuloma venereum agent
Measles virus
Mumps virus
Parainfluenza virus—all types except Parainfluenza virus 3, SF4 strain, which is in Class 1
Polioviruses—all types, wild and attenuated
Poxviruses—all types except *Alastrim, Smallpox, Monkey pox,* and *Whitepox,* which, depending on experiments, are in Class 3 or Class 4
Rabies virus—all strains except *Rabies street virus,* which should be classified in Class 3 when inoculated into carnivores
Reoviruses—all types
Respiratory syncytial virus
Rhinoviruses—all types
Rubella virus
Simian viruses—all types except *Herpesvirus simiae (Monkey B virus)* and *Marburg virus,* which are in Class 4
Sindbis virus
Tensaw virus
Turlock virus
Vaccinia virus
Varicella virus
Vole rickettsia
Yellow fever virus, 17D vaccine strain

C. CLASS 3 AGENTS

1. Bacterial agents:
*Actinobacillus mallei**
Bartonella—all species
Burcella—all species
Francisella tularensis
Mycobacterium avium, M. bovis, M. tuberculosis
Pasteurella multocide type B ("buffalo" and other foreign virulent strains*)
*Pseudomonas pseudomallei**
Yersenia pestis
2. Fungal agents:
Coccidioides immitis
Histoplasma capsulatum
Histoplasma capsulatum var. *duboisii*
3. Parasitic agents:
Schistosoma mansoni
4. Viral, Rickettsial, and Chlamydial agents:
Alastrim, Smallpox, Monkey pox, and *Whitepox,* when used *in vitro*
Arboviruses—all strains except those in Class 2 and 4 (Arboviruses indig-

*See Part V, Footnotes and References, ref. 7.

*USDA permit also required for import or interstate transport.

enous to the United States are in Class 3, except those listed in Class 2. *West Nile* and *Semliki Forest* viruses may be classified up or down, depending on the conditions of use and geographical location of the laboratory.)

Dengue virus, when used for transmission or animal inoculation experiments

Lymphocytic chorimeningitis virus (LCM)

Psittacosis-Ornithosis-Trachoma group of agents

Rabies street virus, when used in inoculation of carnivores (See Class 2)

Rickettsia—all species except *Vole rickettsia* when used for transmission or animal inoculation experiments

*Vesicular stomatitis virus**

Yellow fever virus—wild, when used *in vitro*

D. CLASS 4 AGENTS

1. Bacterial agents: None.
2. Fungal agents: None.
3. Parasitic Agents: None.
4. Viral, rickettsial, and Chlamydial Agents:

Alastrim, Smallpox, Monkey pox, and *Whitepox*, when used for transmission or animal inoculation experiments

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

Herpesvirus simiae (Monkey B virus)

Lassa virus

Marburg virus

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

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

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

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

A. LOW-RISK ONCOGENIC VIRUSES

Rous Sarcoma
SV-40
CELO
Ad7-SV40
Polyoma
Bovine papoilloma
Rat mammary tumor
Avian Leukosis
Murine Leukemia
Murine Sarcoma
Mouse mammary tumor
Rat Leukemia
Hamster Leukemia
Bovine Leukemia
Dog Sarcoma
Mason-Pfizer Monkey Virus
Marek's
Guinea Pig Herpes
Lucke (Frog)
Adenovirus
Shope Fibroma
Shope Papilloma

B. MODERATE RISK ONCOGENIC VIRUSES

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

III. Animal Pathogens (3)

A. ANIMAL DISEASE ORGANISMS WHICH ARE FORBIDDEN ENTRY INTO THE UNITED STATES BY LAW (CDC CLASS 5 AGENT)

1. Foot and mouth disease virus

B. ANIMAL DISEASE ORGANISMS AND VECTORS WHICH ARE FORBIDDEN ENTRY INTO THE UNITED STATES BY USDA POLICY (CDC CLASS 5 AGENTS)

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

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

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

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APPENDIX C—NIH POLICES AND ADMINISTRATIVE PROCEDURES FOR EXPERIMENTS SUBJECT TO THE NIH GUIDELINES

The policies and procedures in this appendix apply only to recombinant DNA research subject to the NIH guidelines (see Pt. I of the guidelines).

These policies and procedures are mandatory for all recombinant DNA research conducted at institutions receiving any funds for recombinant DNA research from NIH. This appendix supersedes previous announcements published in the October 17, 1977, and February 15, 1978, issues of the NIH Guide for Grants and Contracts and previous notices placed in application kits for Public Health Service research and training grants.

This appendix contains NIH policies and procedures in effect as of the date of publication of the final revised guidelines. These policies and procedures can be superseded by subsequent issuances. For current NIH policies, contact the NIH Office of Recombinant DNA Activities (ORDA).

I. *General Requirements.*

A. *Institutional Biosafety Committee (IBC).* Each institution at which recombinant DNA research subject to the guidelines is being conducted must have a standing biosafety committee. Suggestions for the composition of such a committee are discussed under Part IV of the guidelines, which also discusses the roles and responsibilities of principal investigators and institutions. A roster of the members of the Institutional Biosafety Committee (IBC) must be submitted to NIH.

The minimum information must include the names, addresses, occupations, qualifications, and curricula vitae of the chairperson and members of the committee. This information must be submitted to: Office of Recombinant DNA Activities, National Institutes of Health, Room 4A52, Building 31, Bethesda, Md. 20014.

The membership of IBC's is subject to review by ORDA for compliance with recommendations stated in the guidelines. It is the responsibility of each institution to update this information at least annually. As stipulated in the guidelines, ORDA will assist in the formation of an Area Biosafety Committee (ABC) when appropriate. Such a committee will be necessary when additional expertise from outside a given institution is necessary for the IBC to fulfill its functions.

B. *Approval and Registration of Projects.* Central to the implementation of the guidelines is the review of proposed projects by the IBC. When the IBC has approved the project, the experiments may be initiated (except for experiments requiring P4 physical containment, which require prior NIH approval; special procedures cover NIH awards, see sec. II-D below). The institution is responsible for registering approved projects with NIH. ORDA will review approved projects and notify investigators and institutions of the results of such review. (See sec. II-B for requirements for competing and noncompeting NIH applications. See sec. II-E for changes in ongoing projects, and Pt. IV for information on registration.)

II. *Requirements and Procedures for NIH-Supported Projects.* This section describes policies and procedures for projects supported by NIH.

A. *Memorandum of Understanding and Agreement (MUA).* Each application to the National Institutes of Health for a project which involves experiments subject to the NIH guidelines for recombinant DNA research (see sec. I-E of the guidelines) must be accompanied by an MUA prepared in the format shown in the attached illustration. Applicants are urged to follow the sequence and format of the illustration as closely as possible.

An application submitted to NIH without an attached MUA is incomplete and will not be reviewed until a properly executed MUA is provided.

1. *Contents of an MUA.* An MUA must contain the following sections:

a. *Description.* A description of each proposed project, and the name of the individual investigator responsible for the research if other than the principal investigator.

Descriptions must include information on:

(1) source(s) of DNA,

(2) nature of inserted DNA sequences, and

(3) hosts and vectors to be used.

The descriptions must be sufficient to provide information about the experiments without need for reference to other documents. Each performance site must be identified with the name(s) of the organization, city, and State. Ordinarily, no more than two pages of description for each series of experiments are necessary.

b. *Assessment of Containment Levels.* An assessment of the physical and biological containment levels required by the current NIH guidelines for each series of experiments.

c. *Statement by Principal Investigator.* A specific brief statement by the principal investigator agreeing to abide by the provisions of the NIH guidelines and the requirements contained in this appendix concerning shipment and transfer of recombinant DNA materials (see sec. II-F of this appendix).

The principal investigator must also attest to the accuracy of the information in the MUA (see illustration in this appendix).

d. *Information concerning IBC review:*

(1) When facilities are in existence, a certification is required indicating that the IBC has reviewed the facilities and the proposed project(s) and found them to be in compliance with the NIH Guidelines, this Appendix, and other specific NIH instructions. The date of the IBC review must be specified.

(2) When facilities are proposed or are under construction or renovation at the time of the application, an assurance in lieu of a certification must be provided. The assurance indicates that the IBC has reviewed the proposed project and the plans for the facilities proposed or under construction or renovation. The assurance must include a statement that recombinant DNA experimentation will not occur until the completed facility has been reviewed by the IBC and an amendment to the MUA (i.e., the certification described in subparagraph (1)), has been submitted to NIH.

NOTE:Some MUA's may have to incorporate a certification as well as an assurance if there are both certified facilities and facilities under construction or renovation.

e. *Statement Regarding Continuing Compliance.* A statement by the appropriate institutional official that the IBC will insure compliance with the Guidelines throughout the duration of the project.

f. *Signatures.* The signatures of the principal investigator, the IBC chairperson, and the institutional official(s) are required.

g. *Date of MUA.* The date of the MUA for future reference will be the date of the institutional official's signature.

2. *MUA's for Recombinant DNA Research at Multiple Sites.* When research at multiple sites is proposed, the MUA must specify where each part of the project will be carried out. When research is proposed at sites governed by other than the applicant institution, signatures of the appropriate officials at both the applicant institution and the institution(s) where the recombinant DNA research is to be conducted are required. The signatures shall indicate that the IBC's of each of the institution(s) where the research is to be performed have given the certification and/or assurance required in item d of the MUA.

3. *MUA's Associated with Individual Fellowship Applications, Research Career Development Award Candidates (RCDA), Research Career Awardees (RCA), and Institutional National Research Service Fellowship Applications.* When projects involving recombinant DNA technology subject to the Guidelines are involved, fellowship applicants, RCDA candidates, RCA's, or Program Directors for Institutional National Research Service Fellowship Applications should attach to the application either a copy of MUA(s) already submitted to the NIH, or submit a new MUA(s) as indicated below. If a copy is submitted, the fellowship applicant, RCDA candidate, RCA, or Program Director (if other than the principal investigator) must sign the MUA copy under the signature of the principal investigator.

If any recombinant DNA work is proposed other than that indicated in an existing MUA(s), a new or amended MUA must be submitted to NIH in accordance with procedures in II-B and II-E of this Appendix. These procedures also apply to experiments using recombinant DNA technology in courses supported by an institutional fellowship.

B. *Submission of Memorandum of Understanding and Agreement (MUA).*

1. *Competing Applications.* For competing applications involving recombinant DNA research subject to the Guidelines, an MUA must be submitted to the Division of Research Grants, NIH, with the application.

2. *Noncompeting Applications.* Each noncompeting continuation application involving recombinant DNA research subject to the Guidelines must be accompanied by an updated MUA that indicates that the IBC has reviewed the project prior to submission of the application, and has found it still in compliance with NIH Guidelines.

IMPORTANT NOTE: If an investigator wishes immediately to initiate a project after IBC approval, after submitting a new competing application to NIH, the procedures for registration of non-NIH projects, described in section IV of this Appendix, must also be followed. If an investigator wishes immediately to initiate new experiments in an ongoing project, an MUA must be filed with NIH within 30 days of IBC approval, even if the proposed experiments are described in an MUA submitted with a noncompeting or a competing renewal application. In the latter cases, see procedures in section II-E of this Appendix, dealing with changes in ongoing projects, which must also be followed.

C. *Notation on Applications for Research and Training Grants.* NIH application forms will be revised to include a block to be checked indicating whether recombinant DNA research subject to the Guidelines is involved. Until such time as revised forms are available, applicants should specify in capital letters at the bottom of the first page of the application the following statement:

APPLICATION INVOLVES EXPERIMENTS SUBJECT TO GUIDELINES FOR RECOMBINANT DNA RESEARCH

D. *Award Procedures.* Prior to award, MUA's will be reviewed by ORDA for compliance with the requirements of the Guidelines. Notification of the status of NIH review of the MUA will be accomplished by one of the following 3 footnotes on the Notice of Grant Award:

Footnote 1. "Protocols in MUA dated —/—/— conform to standards of NIH Guidelines."

Footnote 2. "Protocols in MUA dated —/—/—, as modified, conform to standards of NIH Guidelines."

Footnote 3. "Protocols in MUA dated —/—/— do not conform to standards of NIH Guidelines."

Footnote 1 will be used to indicate that the MUA has been reviewed by ORDA, and the protocols have been found to conform to the standards set forth in the Guidelines.

Footnote 2 will be used to indicate that the MUA has been reviewed by ORDA and that certain aspects of the MUA do not conform to standards set forth in the Guidelines. Use of this footnote indicates that NIH has notified the institution that appropriate action must be taken to bring the protocols in question into compliance with NIH standards, or that the protocols in question must not be carried out.

Footnote 3 will be used to indicate that the MUA has been reviewed by ORDA and the protocols have been found not to conform to standards set forth in the Guidelines. This footnote indicates that the proposed experiments are not to be carried out until they have been brought into compliance with NIH standards, and a revised MUA has been submitted to the NIH.

E. *Changes in Ongoing Projects.* Changes in a project subject to the Guidelines must be reported to the IBC by the principal investigator.

1. *Protocols for Which Containment Levels are Explicitly Specified by Guidelines.* The procedures described in this section apply only to research projects for which containment levels are explicitly stated in the Guidelines or in announcements from ORDA, except for projects requiring P4 physical containment, which require NIH approval prior to initiation of the research.

Depending upon the judgment of the IBC as to whether the proposed changes are major or minor, the procedures described in a and b below are to be followed.

a. *Major Changes.* A new or revised MUA is required for introduction of recombinant DNA research subject to the guidelines into an ongoing project, or for significant changes in the recombinant DNA aspects of an ongoing project. Examples of changes in an ongoing recombinant DNA project which would require the filing of a new or amended MUA would include (i) significant changes in hosts or vectors. (ii) significant changes in the donor species or the nature of the DNA segment being selected, (iii) major changes in the physical location of the experiments, or (iv) a change of the investigator responsible for the conduct of the experiments.

For such changes in an ongoing project, a new or revised MUA must be submitted to the IBC. Once IBC approval has been obtained for other than projects requiring P4 containment, the experiments may be initiated. The institution must then forward the MUA directly to the program administrator of the awarding NIH Bureau, Institute, or Division within 30 days of approval by the IBC. The MUA must identify the project by grant number. The principal investigator will be notified by letter regarding the NIH review of the new or revised MUA. If review by ORDA finds that an MUA, or certain aspects of an MUA, do not conform to standards set forth in the guidelines, the principal investigator and the IBC will be notified. In such cases, immediate action must be undertaken to bring the protocols into compliance with NIH standards or the experiments in question must be suspended.

b. *Minor Changes.* Principal investigators should submit information on

minor changes in research protocols to the IBC. Minor changes need not be reported to NIH until an updated MUA is required (see section II-B of this appendix).

2. *Protocols for Which Containment Levels are Not Explicitly Specified by the NIH Guidelines.* Investigators who wish to initiate recombinant DNA experiments for which containment levels are not explicitly stated in the guidelines or in announcements from ORDA must obtain approval from the NIH prior to initiating the proposed experiments.

Because it is anticipated that the setting of containment levels for this class of experiments will require review by the Recombinant DNA Advisory Committee, investigators are strongly urged to provide to ORDA in writing full information on the proposed experiments prior to submission of a formal MUA.

F. *Shipping Requirements.*

NOTE.—Requirements regarding shipment and transfer of recombinant DNA materials may be incorporated by reference into the MUA (see illustration in this appendix).

All MUA's must indicate that the principal investigator (program director, fellow, or candidate) agrees to comply with the following provisions pertaining to shipment or transfer of recombinant DNA materials:

1. Prior to shipment or transfer of recombinant DNA materials within the United States, the sending laboratory shall obtain a written statement from the requesting laboratory that:

a. Research involving recombinant DNA molecules shall be conducted in compliance with NIH guidelines, this appendix, and other NIH instructions, and

b. The project proposed by the requesting laboratory has been reviewed and approved by the IBC of the requesting laboratory.

2. Prior to shipment of recombinant DNA materials to a country other than the United States, the sending laboratory shall obtain a statement from the requesting laboratory stating that research involving recombinant DNA molecules shall be conducted in accordance with the containment levels specified by the NIH guidelines, or applicable national guidelines if such have been adopted by the country in which the research is to be conducted.

3. The sending investigator shall maintain a record of all shipments of recombinant DNA materials.

NOTE.—See section II-D-3 of the guidelines for restrictions on distribution of certified host-vector systems.

III. *Policy and Procedures for Recombinant DNA Research Supported by NIH and Conducted in Foreign Countries.*

A. *Policy.* Many countries in which NIH-supported recombinant DNA research may be conducted have adopted guidelines for the conduct of this research which are either comparable to or based on principles similar to those of the NIH guidelines for recombinant DNA research. Also, many countries have existing organizations and procedures to review and register recombinant DNA research, and require documentation similar to that of NIH. If such guidelines and procedures exist and are comparable to the NIH guidelines, review and approval by the appropriate national body in the country in which the research is to be conducted, in general, will be accepted as assurance that the research will be conducted in a responsible manner and in accord with national guidelines. However, NIH reserves the right to withhold funding if the safety practices to be employed are not reasonably consistent with the NIH guidelines.

B. *Requirements and Procedures for NIH-Supported Research in Countries with Guidelines.* The following procedures apply both to NIH awards in foreign countries and to U.S. investigators intending to conduct recombinant DNA research with NIH support in foreign countries which have adopted guidelines. For applications for NIH awards in foreign countries, the required documentation (see below) may be included with the application, or may be submitted at a later date, but must be submitted prior to the time of award. For U.S. investigators intending to conduct recombinant DNA research abroad, the documentation should be submitted prior to initiation of the project.

For all NIH-supported research abroad using recombinant DNA technology, a document providing the following information must be submitted to NIH:

1. A description of each proposed project and the individual investigator responsible for the research, if other than the principal investigator. Descriptions should indicate the sources of DNA, nature of inserted DNA sequences, hosts and vectors to be used, and must be of sufficient detail to provide information about the research without need for reference to other documentation. Ordinarily, no more than two pages of description for each series of experiments are necessary.

2. An assessment of the level(s) of physical and biological containment required by the guidelines of the country in which the research is to be conducted.

3. Documentation that the research project is in compliance with the applicable national guidelines and, where required, has been registered with the appropriate national body in the country in which the research is to be conducted. If approval of the national body is required prior to initiation of the research, the documentation must indicate that such approval has been received.

4. The signatures of both a responsible official and the principal investigator.

5. The date of signature of the responsible official. This will become the date of the document for future reference.

C. *Requirements Regarding Countries Which Have Not Adopted Guidelines.* NIH funds may not be used for the conduct of recombinant DNA research in a country which has not adopted national guidelines, unless the research is in full compliance with NIH Guidelines and the procedures required for U.S. grant applications have been fulfilled—i.e., establishment of an institutional biosafety committee, filing of an MUA, etc.

IV. *Registration of Recombinant DNA Projects.*

A. *NIH-Supported Projects.* An NIH-approved MUA will constitute registration of projects awarded by NIH. For immediate initiation of projects pending with NIH see "Important Note" under section II-B of this appendix.

B. *Required Registration of Non-NIH Projects.* The guidelines require that institutions receiving NIH funds for recombinant DNA research shall inform NIH of all initiated recombinant DNA projects subject to the guidelines (see section IV-C-1 of the guidelines). For these projects, the required information must be submitted within 30 days of the IBC's approval.

C. *Voluntary Registration of Non-NIH Projects.* The guidelines stipulate that any institution which is not required to comply with the guidelines may nevertheless register with the NIH ongoing projects involving recombinant DNA research subject to the guidelines (see section IV-C-2 of the guidelines).

D. *Procedures for Registration.* The procedures in this section apply to registration of projects in B and C above. Because non-NIH projects filed with the NIH will immediately be added to the register of ongoing recombinant DNA research, institutions may register only those projects which are ongoing or about to be initiated. Institutions should not submit pending projects to this register.

Institutions must register these projects directly with ORDA. It is the responsibility of the institution to insure that this information is accurate and up-to-date by submitting any necessary revised information on a timely basis. Institutions are responsible for notifying ORDA when a project is completed or terminated so that it may be removed from the register.

For registration of projects described in B and C above, the following information must be submitted:

1. A short title for the project.
2. Registry number of project.
3. Start and end dates of the project.
4. The name of the principal investigator and the name and address of the institution, including the department in which the research is being conducted and the location of the project.
5. The source of support for the project.
6. A description of each project and the name of the investigator responsible for the research. Descriptions should indicate information on the sources of DNA, nature of inserted DNA sequences, and the host-vector systems being used. Ordinarily, no more than two pages of description for each series of experiments are necessary.
7. An assessment of the level(s) of physical and biological containment required by the Guidelines for each series of experiments.
8. A statement that an Institutional Biosafety Committee has reviewed the project and found it to be in compliance with the NIH Guidelines.
9. The signatures of the principal investigator, the chairperson of the IBC, and the institutional official.
10. The date of signature by the institutional official. This date will be used for reference purposes.

E. *Review.* Institutions will be notified in writing of ORDA's review of the information submitted for registration of recombinant DNA projects not supported by NIH. If review by ORDA finds that the protocols do not conform to standards set forth in the Guidelines, the institution is expected to take appropriate action to bring the protocols into compliance with NIH standards.

V. *Lowering of Containment Levels for Purified DNA and Characterized Clones.*

A. *Purified DNA Other Than Plasmids, Bacteriophages, and Other Viruses.*

The Guidelines stipulate that the formation of DNA recombinants from cellular DNA's that have been purified and which are free of harmful genes can be carried out under lower containment conditions than those required for the corresponding shotgun experiment. IBC approval is sufficient for such a reduction of containment level, except for (i) primate DNA, which also requires prior NIH approval, or (ii) any lowering of containment to levels below P1+EK1, which also requires prior NIH approval (see sec. III-A-3-a of the Guidelines).

The IBC must notify ORDA in writing of all such actions. Many of these actions will be in the context of submission of a new or revised MUA.

B. *Characterized Clones of DNA Recombinants.* The Guidelines permit IBC's to give approval for a single-step reduction in physical or biological containment on receipt of evidence of characterization of a clone derived from a shotgun experiment and its probable freedom from harmful genes (see sec. III-A-3-b of the Guidelines). IBC approval is sufficient for such a reduction except for (i) primate DNA which requires prior NIH approval, or (ii) any lowering of containment to levels below P1+EK1, which also requires prior NIH approval. The IBC must notify ORDA in writing of all such actions. Such notification must clearly indicate the name of the principal investigator and the project supporting the work.

Reduction of containment levels by more than one step, use of primate DNA, or cases involving lowering of containment to levels below P1+EK1 require prior approval by NIH. In the latter cases, complete information must be submitted to ORDA. Many of these cases are expected to require review by the RAC at its next scheduled meeting.

VI. *Exceptions and Exemptions to the Guidelines.*

A. *Exceptions.* Exceptions to prohibited experiments require express approval by the Director, NIH, on recommendation of the RAC after appropriate notice and opportunity for public comment (see sec. I-D of the Guidelines).

B. *Exemptions.* Classes of exempt experiments, which are not covered by the Guidelines, are cited in section I-E of the Guidelines. It is anticipated that additional exemptions for specific experiments which fall into the categories cited in sections I-E-4 and I-E-5 of the Guidelines may be granted by the Director, NIH, on the recommendation of the RAC and after appropriate notice and opportunity for public comment.

C. *Requests for Exceptions and Exemptions.* Requests for an exception to a prohibition or an exemption from the Guidelines must be submitted to ORDA accompanied by adequate documentation. Because the handling of requests for exceptions and exemptions will probably be a time-consuming process, investigators are strongly urged to discuss the proposed request with the staff of ORDA by telephone in advance of submission of a formal request. Decisions or requests for exceptions or exemptions will be published in the *Recombinant DNA Technical Bulletin.*

Illustration

NAME OF INSTITUTION

MEMORANDUM OF UNDERSTANDING AND AGREEMENT

Application/Grant Number (if applicable) _____

Investigator's Telephone Number _____

Description

(To be supplied)

Assessment of Levels of Physical and Biological Containment

(To be supplied)

The information above is accurate and complete. I am familiar with and agree to abide by the provisions of the current NIH Guidelines and other specific NIH instructions pertaining to the proposed project. I agree to comply with the requirements specified in Appendix C pertaining to shipment and transfer of recombinant DNA materials.

_____
Principal Investigator Date

I certify that the Institutional Biosafety Committee (IBC) has reviewed on (date) the proposed project for recombinant DNA experiments, and has found it to be in compliance with NIH Guidelines and other specific NIH instructions pertaining to the proposed project.

AND/OR

I assure that the IBC has reviewed on (date) the proposed project and the plans for facilities proposed or under construction or renovation. Recombinant DNA experimentation will not occur until the completed facilities have been reviewed by the IBC and an MUA with certification has been submitted to the NIH.

I agree that the IBC shall monitor throughout the duration of the project the facilities, procedures, and training and expertise of the personnel who are working on the project and for the facilities.

_____
IBC Chairperson Date

_____
Applicant Institutional Official Date

_____
Institutional Official Date
(Additional Performance Sites, if applicable)

NATIONAL INSTITUTES OF HEALTH, ENVIRONMENTAL IMPACT ASSESSMENT OF A PROPOSAL TO RELEASE REVISED NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES

PREPARED BY THE OFFICE OF THE DIRECTOR, NATIONAL INSTITUTES OF HEALTH—JULY 1978

SUMMARY

Nature of Document: Environmental Impact Assessment.

Agency: National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

Type of Action: (X) Administrative. ( ) Legislative.

Description of Action: Publication for public comment of proposed revised guidelines for research involving recombinant DNA molecules.

Organization of Material: Background information is presented on the recombinant DNA process and on the presumed risks and demonstrable benefits of this basic research technique. Next the proposed revised guidelines are analyzed according to their four main parts: scope, principles of containment of possibly hazardous agents, proposed changes in the containment for experiments to be covered by the revised guidelines, and roles and responsibilities of investigators and institutions.

Analysis of Alternatives: For each of these four main parts, the assessment is presented under four sections: analysis of the current Guidelines (in effect since June 23, 1976); alternatives (revisions) proposed by the NIH Recombinant DNA Advisory Committee (RAC) [FEDERAL REGISTER, September 27, 1977]; alternatives proposed by the Director, NIH, after full consideration of scientific evidence, public comments, and the testimony taken in a 2-day meeting of the Director's Advisory Committee (DAC) at which scientists of various disciplines, representatives of environmental groups, and other witnesses discussed the RAC proposals; and finally, the projected environmental impact of research to be conducted under the NIH Director's proposed guidelines. Appendix A will aid in comparing the containment levels under the current guidelines with those under the two alternatives. Appendix B shows how those alternatives would have affected all NIH-funded recombinant DNA experiments active in December 1977.

Environmental Impact of the Proposed Action: As can best be determined from all evidence compiled to date and analyzed in numerous scientific and public forums, there will be no adverse environmental impact from recombinant DNA research conducted under the Director's proposed revisions. The *Environmental Impact Statement on NIH Guidelines for Research Involving Recombinant DNA Molecules,* issued in October 1977, predicted that the environmental impact of research conducted under the 1976 NIH Guidelines would be the continued protection of the laboratory worker, the general public, and the environment from conjectural hazards. So far, this prediction has been confirmed: We know of no scientists conducting recombinant DNA research in the United States or other countries who are not following the NIH or comparable guidelines, and no untoward effect of the research has been reported. Meanwhile, new scientific evidence as well as extensive experience in operating under the NIH Guidelines indicate that revisions are in order. The predictable effect of continued use of recombinant DNA techniques under the Director's proposed revisions would be a greater realization of the benefits of this valuable tool without compromise of safety.

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FOREWORD

In June 1976 the National Institutes of Health, with the concurrence of the Secretary of Health, Education, and Welfare and the Assistant Secretary for Health, issued guidelines to govern the conduct of NIH-supported research involving recombinant DNA molecules. These guidelines stated that they would be "subjected to periodic review (at least annually) and modified to reflect improvements in our knowledge of the potential biohazards and of the available safeguards." Since that time, a number of scientific, administrative, and legislative events have occurred that should be summarized at the outset, for they are reflected in the revisions of the NIH guidelines as proposed, first by the Recombinant Advisory Committee and currently by the Director, NIH.

Recombinant DNA experiments have proceeded in hundreds of laboratories throughout the world. The subject has been discussed and debated in countless meetings, and the public has been consulted as well as the scientific community. NIH has taken into account public comments in preparing the original guidelines, an environmental impact statement (EIS), and the proposed Director's revision.

One of the most important recent developments has been the careful scrutiny, from a very broad point of view, of the premises upon which the original guidelines were based. Thus, the molecular biologists, who first raised questions about the safety of recombinant research, have now had greater opportunity to consider their concerns in the company of many experts on infectious disease, epidemiology, viruses, plants, laboratory safety practices, ecology, and other relevant disciplines.

From all of these activities have emerged certain important facts. For one, no evidence has come to light that any of the thousands of individual recombinant DNA clones construct-

ed over the last 5 years have yielded a product harmful to man or the environment. On the other hand, many examples of useful knowledge obtained through such techniques continue to accumulate rapidly.

Scientific developments

No scientific evidence not considered in the promulgation of the guidelines has emerged to support the fears that the use of these techniques will create a harmful product. On the contrary, scientific information has been developed over the past 2 years that lessens concern over the possible environmental hazard. Dr. Roy Curtiss, professor of microbiology at the University of Alabama School of Medicine in Birmingham, and others have demonstrated that biological containment measures—methods developed to weaken bacteria used in the experiments—do prevent these bacteria from surviving in a natural environment, and would do so if they escaped from the laboratory.

While the probability of doing harm with laboratory recombination of genes has not been, and never will be, reduced to zero, we have reached a point where the burden of proof is shifting toward those who would restrict such activities. The careful interpretation of evidence obtained before and after June 1976 has reduced to inconsequential levels the probabilities that *E. coli* K-12, the host most used in recombinant DNA experiments, can be converted to an epidemic pathogen. Much of the relevant data and their discussion by experts are now available in the published proceedings of an NIH-sponsored meeting in Falmouth, Mass., on June 20-21, 1977 (Journal of Infectious Diseases, May 1978).

The Falmouth conference brought out evidence that the risk of transforming *E. coli* K-12 into a pathogen is minimal, either for laboratory personnel or the public at large. Dr. Sherwood Gorbach, chairman of the conference, has reported that there was scientific consensus on this matter among all in attendance, including microbiologists who work with disease-producing bacteria.

Much of the concern expressed about recombinant DNA experiments relates to the creation of novel organisms in the laboratory. Additional evidence, however, suggests that the recombinations of DNA produced in the laboratory may be very similar to many that occur in nature. If further work confirms and extends this evidence, then the concern about creating novel forms of life will be put into a new perspective.

Administrative developments

Implementation of the NIH guidelines

The current NIH guidelines provide not only explicit instructions about the conduct of experiments, but also an administrative framework for their implementation. They set out the respective responsibilties of the principal investigator, the institution where the work is conducted (including the institutional biohazard committee), the NIH Recombinant DNA Advisory Committee (or simply "Recombinant Advisory Committee" or "RAC," the technical body responsible for proposing the guidelines), and the NIH staff.

The NIH Office of Recombinant DNA Activities (ORDA) was established to coordinate the administration of NIH policies and procedures for safe utilization of recombinant DNA technology in research. Dr. William Gartland is Director of ORDA. Over the 2 years the implementation of the guidelines by participants in this research has proceeded well. Approximately 130 institutions where NIH-supported research is taking place have established institutional biohazard committees, and approximately 350 projects are involved.

Over the past 2 years administrative practices have evolved to deal with requirements of the guidelines. One of the requirements is a means for interpretation. The standards in the guidelines are very explicit about the conduct of permissible experiments. Still, questions of interpretation continue to arise and must be dealt with. Our determination to assure that the experiments comport with the standards of the guidelines has necessitated a number of administrative delays in acting on research protocols.

Another area of difficult administration has been certification of new host-vector systems. These represent microorganisms weakened by various methods to prevent their survival were they to escape from their specially contained environment in the laboratory. Under the current guidelines, the Recombinant Advisory Committee must review all applications for new host-vector systems and recommend for certification those that meet the relevant criteria.

Undoubtedly the presence of the guidelines and their implementation have caused some experiments to be postponed and some scientific work to be delayed. At the same time, NIH, having embarked upon this conservative course, believes it must guarantee the integrity of the administrative safeguards and see that due process is observed in implementation.

Policy issues

Three key policy issues concerning recombinant DNA research have dominated NIH attention during the administration of the NIH Guidelines. They are the determination of the environmental impact, if any, of the NIH Guidelines and the research conducted thereunder; the patenting of recombinant DNA research inventions developed under Federal support; and the extension of the NIH Guidelines nationally through existing regulation or new legislation. Discussions of these policy issues follow:

National Environmental Policy Act (NEPA). In accordance with the National Environmental Policy Act of 1969 (NEPA), NIH undertook an environmental impact assessment of environmental effects, if any, of the original NIH Guidelines. A draft environmental impact statement was published in the FEDERAL REGISTER in September 1976 for public review and comments. On the basis of the comments received, NIH published a final EIS in October 1977.

During the development of the EIS, two suits under NEPA were brought against the Department. One suit, in the Federal District Court in New York, alleged that NIH failed to comply with NEPA by not completing an EIS before supporting recombinant DNA research and before releasing the original guidelines. The Government has answered the allegations, and the case is pending in New York.

The second suit filed in the Federal District Court in Washington, D.C., by a resident of Frederick, Md., sought an injunction to prevent NIH from conducting a risk-assessment experiment at the Frederick Cancer Research Center without first filing an environmental impact statement. On February 23, 1978, a decision was rendered by the U.S. District Court against the issuance of an injunction, and this decision was affirmed by the U.S. Court of Appeals on March 8, 1978. It was the decision of the court that recombinant DNA research was beneficial, that the experiment under question was important, and that it posed no substantial risk. The court went on to state that "the Recombinant DNA Research Guidelines represent an effort by many scientists to evaluate the hazards and provide safe methods for their control. The record reflects that NIH has carefully considered the potential risks of this experiment under the guidelines and has taken the necessary precautions * * *. The EIS does represent a 'hard look' by NIH at recombinant DNA research performed in accordance with its guidelines. It appears that compliance with the NIH guidelines will insure that no recombinant DNA molecules will escape from the carefully controlled laboratory to the environment."

Patent Policy. Shortly before release of the NIH Guidelines in June 1976, NIH received a letter from Stanford University noting that both Stanford and the University of California were

applying for patent protection for recombinant DNA inventions developed by their NIH-supported investigators, and asking NIH to review relevant DHEW policies. In view of the intense public interest in this research generally, the two universities felt the need for a formal advisory opinion on the patenting of such inventions developed under NIH grants or contracts. A number of other universities indicated similar interest in obtaining the official NIH view.

Prior to making an official pronouncement of DHEW-NIH policy with respect to patenting of recombinant DNA research inventions, NIH decided to solicit comments from a broad range of individuals and institutions. An analysis of the comments received was completed in December 1976 and was referred to the Federal Interagency Committee on Re- combinant DNA Research.

When the guidelines were released in June 1976, a key public issue was their extension to the rest of the public and private sectors. Commentators whose views were solicited agreed that there must be standards to govern recombinant DNA research and that the NIH Guidelines could provide the standards for such research nationally. They were divided, however, on whether to approach that goal through the use of patent agreements. They noted that the implementation of the NIH Guidelines through licenses granted under patents would be awkward at best and only a partial solution.

The Interagency Committee members reviewed the matter in April and May of 1977. Most members voiced strong support for DHEW policies governing Institutional Patent Agreements (IPA's), and all except representatives of the Department of Justice believed that recombinant DNA inventions should be considered within the existing terms of the IPA. The Justice Department opinions rested heavily on a draft bill originally proposed by Senator Kennedy for the regulation of recombinant DNA research activities. Specifically, Justice referred to the patent sections of this bill that were based on the concept of Government ownership of recombinant DNA research inventions. In subsequent versions of this bill, however, all references to patents were eliminated.

On the basis of the review by the Interagency Committee and by the Assistant Secretary for Health and the General Counsel for DHEW, it was decided that, at least for the present, recombinant DNA research inventions developed under DHEW-NIH support should continue to be administered within current DHEW patent agreements with universities. Each agreement, however, will be amended to permit the institution to grant a license under patents secured on any such invention only if the licensee provides assurance of compliance with the physical and biological containment standards set forth in the guidelines. Thus, the requirements set for NIH grantees and contractors will thus be honored by licensees as well.

Legislative Developments. The Federal Interagency Committee on Recombinant DNA Research recommended in March 1977 that legislation be passed to extend the standards of the NIH Guidelines to all recombinant DNA activities in the public and private sectors. On the basis of the recommendations, legislation was developed under HEW Secretary Joseph A. Califano, Jr., and an Administration bill was introduced in the Congress. The bill was considered in Congressional hearings; other bills on the subject were introduced. After several redrafts by the relevant subcommittees during 1977, a Senate bill was reported to the Floor and a House bill was reported to the full Committee.

The two bills reported out contained many elements of the original Administration bill. A number of differences emerged, however, that would necessarily involve a greater administrative burden and some further delays and duplication in handling the highly technical matters involved in standard-setting and monitoring.

Pending legislation introduced in 1978 provides the most promising solution yet available for establishing national standards for the use of recombinant DNA techniques. The bill H.R. 11192 was reported by the Committee on Interstate and Foreign Commerce on March 24, 1978. It is an interim 2-year measure that provides for sensible regulation and public oversight. It was referred to the Committee on Science and Technology, was considered by its Subcommittee on Science, Research and Technology for a period of 21 days, and was reported from the Committee for House action on April 21. H.R. 11192 reflects new scientific information and administrative developments since the release of the NIH Guidelines in June 1976. The general administrative structure of the guidelines and the standards for biological and physical containment are endorsed for purposes of regulation. Thus, flexible regulation with national standards is the intent of the bill.

International activities

During the legislative hearings on recombinant DNA research, a number of questions were raised concerning international activities in this field. In light of this interest, the Federal Interagency Committee, at the request of representatives from the State Department, undertook a review of activities in other countries. This review was the basis for a Committee report issued in November 1977 on recombinant DNA activities throughout the world, with recommendations for fostering common safety standards. Scientists abroad, as in the United States, have played a major role in bringing potential hazards of recombinant DNA research to the attention of scientists, governments, and international organizations.

The issue of recombinant DNA research has been studied by national and international bodies. In many cases some form of control has been adopted, but nowhere has the research been totally banned. The United Kingdom, Canada, France, the Federal Republic of Germany, and the Soviet Union have issued guidelines that differ in detail but are similar conceptually to the NIH Guidelines. Other countries are generally following the NIH or U.K. Guidelines, including Denmark, the Netherlands, Israel, Sweden, and Switzerland. The European Science Foundation (ESF) has endorsed the U.K. Guidelines; the European Molecular Biology Organization (EMBO) has endorsed use of either the U.K. or the NIH Guidelines; and the International Council of Scientific Unions (ICSU) and the World Health Organization (WHO) have urged nations to adopt the principles that these two sets of guidelines embody.

As of the summer of 1977, there were an estimated 150 research projects using recombinant DNA techniques under way in Europe, 300 in the United States, and perhaps 20-25 altogether in Australia, Japan, and the Soviet Union. All appear to be conducted under some form of safety practices and procedures.

A number of national and international activities foster the monitoring of recombinant DNA research for purposes of safety and health. In the United Kingdom, the government's health and safety executive will be responsible after October 1978 for insuring that the standards of the United Kingdom Genetic Manipulation Advisory Group (GMAG) are followed in matters relating to safety of employees and the general public. The GMAG, consisting of representatives from the scientific, public, and private sectors, reviews recombinant DNA research projects for conformance to appropriate safety standards and practices. Similar advisory groups have also been established in other European countries, and efforts are underway to identify appropriate governmental bodies to insure compliance with GMAG standards.

Proposed Revised Guidelines

The RAC-proposed revisions

In December 1975 the Recombinant Advisory Committee recommended proposed guidelines for review and decision by the Director, NIH. To assist in the review, a special meeting of the Advisory Committee to the Director, NIH, was convened in February 1976. Members of the committee represented not only science but such other disciplines as law, ethics, and consumer affairs. Comments received from committee members and a number of public witnesses represented a wide range of views. A number of issues were referred back to the Recombinant Advisory Committee for their comments in April 1976. On the basis of all the comments received and the responses of the RAC, the NIH guidelines were finalized by the Director, NIH, and released in June 1976 along with an extensive "Decision document."

In 1977 the Recombinant Advisory Committee, in accordance with its mandate in the original guidelines, began the process of proposing revisions to them. A subcommittee of the RAC held open meetings in March and April. Following this, the proposed revisions were considered and revised by the full committee at open meetings in May and June. On September 1, 1977, the RAC's proposed revised guidelines were referred to the Director, NIH, for consideration and decision.

These proposed guidelines were published in September for comment in the NIH Recombinant DNA Technical Bulletin. The bulletin is a new NIH publication that links recombinant DNA investigators in the United States and abroad with the advisory groups and organizations active in this field. To provide further opportunity for public comment, the proposed revised guidelines were published in the FEDERAL REGISTER on September 27, 1977 (42 FR 49596). The revisions proposed by the RAC are described in detail in the following assessment, but the key changes can be summarized as follows:

Definition. A new definition was proposed to cover only "novel recombinant DNA"—namely, DNA segments from species not known to exchange chromosomal DNA by natural physiological processes. Accordingly, a class of recombinant DNA, to be exempt from the guidelines, would have to appear on a list of "non-novel exchangers."

Physical Containment. revisions here incorporated the philosophy and guidance of the report by NIH and the European Molecular Biology Organization (EMBO) on the requirement of physical containment. A number of revisions were also made in the organization of this section.

Biological Containment. This section was expanded from the 1976 guidelines to include (1) a new nomenclature—HV1, HV2, and HV3—incorporating a variety of host-vector systems into the framework initially established for *E. coli* K-12; (2) a more restrictive set of requirements for HV3 host-vector systems; and (3) a new section describing mechanisms for certification of host-vector systems.

Prohibited Experiments. A major recommendation would allow the Director, NIH, to exercise discretion in permitting exceptions to the prohibited experiments, as in studies of risk assessment.

Permissible Experiments. On the basis of the scientific evidence on the safety of *E. coli* K-12, some categories of experiments were classified at lower containment levels. Several other categories, however, remained the same as in the current guidelines. For certain categories, discretion was permitted by the investigator, and some categories of experiments were exempted.

Implementation. Several changes were recommended in the responsibilities for the local institution. The recommendations included requirements for training of research personnel, criteria for determining the need for medical procedures, clarification of membership on institutional bio- hazards committees, and the requirements for a biological safety officer where work is being done at the P3 and P4 levels.

The NIH-proposed revisions

The proposed revisions by the Recombinant Advisory Committee were published in the FEDERAL REGISTER for comment on September 27, 1977 (42 FR 49596), and notice of a public hearing was published in the FEDERAL REGISTER on November 22, 1977 (42 FR 59918). In December the RAC-proposed revisions were considered by the Advisory Committee to the Director, NIH (DAC) at a hearing attended by scientific experts and public witnesses. As at the 1976 hearing of the DAC, the membership was augmented to assure that the committee's perspectives included sufficient expertise and opinion on relative scientific, environmental, occupational, and public policy issues. In addition, special arrangements for the meeting included inviting 12 witnesses to represent industrial research, academic research, labor, and environmental groups. A number of others requested the opportunity to contribute their views and testified at the hearing.

Members of the Recombinant Advisory Committee attended to explain the proposed revisions, and members were present from the Federal Interagency committee on Recombinant DNA Research which represents all Federal departments and agencies that support or conduct such research or have regulatory authority in this area.

Since September 1977, when the proposed revisions were published in the FEDERAL REGISTER, they have been reviewed in light of the many comments received from the public and the scientific community. The public hearing provided a forum for all points of view, especially from the environmental perspective. On the basis of the analysis of the hearing and the correspondence, the following general points emerged:

- The recommendations by the Recombinant Advisory Committee were generally supported. There was universal sentiment for giving the Director, NIH, discretion to exempt certain experiments from the provisions of the guidelines, especially when this would permit knowledge to be gained bearing on the provisions themselves. There was overwhelming sentiment for exempting from the guidelines experiments involving most "self-cloning" systems, as well as pairs of harmless organisms that transfer genes in nature. That is, many of the experiments currently classified at the P1 + EK1 level should be exempted from the guidelines. And witnesses from the scientific community strongly advocated further consideration of revisions involving work with viruses and plant pathogens.
- On the basis of these suggestions, a "Joint United States-EMBO Workshop to Assess Risks for Recombinant DNA Experiments Involving the Genomes of Animal, Plant, and Insect Viruses" was convened on January 26-28, 1978, in Ascot, England. The report of the workshop (see appendix E) was reviewed by a working group sponsored by NIH on April 6-7, and the recommendations from that group (see appendix F) were referred to the Recombinant Advisory Committee. The committee, in turn, lent their endorsement in recommendations at a meeting held at NIH on April 27-29. The analysis of existing knowledge of viruses by the groups of experts indicate that the risks of cloning viral DNA in a bacterium like *E. coli* is not greater, and is usually much less, than the risk of handling the parent virus itself.
- Further, a meeting of agricultural scientists was convened on March 20-21, 1978, under the joint sponsorship of the Department of Agriculture, the National Science Foundation, and the National Institutes of Health. Their recommendations were also reviewed by the Recombinant Advisory Committee at their April meeting. It was the conclusion of the agricultural scientists that containment conditions for incorporation of DNA from plant pathogens into *E. coli* K-12 were unjustifiably high.

● International scientific representatives present at the hearing reported on guidelines prevailing in the United Kingdom and Western Europe and on their interpretation by Genetic Manipulation Advisory Groups. It was pointed out that some experiments are permitted in Europe which are not permitted in America. More importantly, it was noted that there is no factual basis upon which to defend the greater stringency of the U.S. (NIH) guidelines.

● There was special emphasis by public commentators on the need for procedures at the local and national level to insure public participation and oversight. The public and scientific commentators were especially concerned that there should be a commitment at the local and national levels to the training of all laboratory personnel, and to health surveillance, when feasible, to insure occupational health and safety. Concern was also expressed for local community participation to insure that practices in the laboratory meet public and environmental safety requirements.

● Several representatives from the private sector urged that NIH consider introducing mechanisms into the proposed revisions to allow private-sector participation through the guidelines. They urged that NIH provide for their voluntary registration, certification of their host-vector systems, and provision for the protection of proprietary information and patent rights.

● Strong support came from both the scientific community and the public for clear enunciation of the benefits and potential risks of this research. In addition, several of the public commentators urged that the rationale for the classification of permissible experiments be stated more clearly.

● Finally, a number of commentators in the scientific community and representatives from institutional biohazards committees advocated more flexible implementation of the NIH guidelines. Specifically, the locus of responsibility for the use of the guidelines must shift further toward the institutions conducting the research. Present requirements for NIH approval before an experiment may proceed have caused delays unjustified by proof that safety has been enhanced.

All of the issues raised by the commentators were carefully analyzed. A number of possible revisions were developed and referred to the Recombinant Advisory Committee for review. The following items were among those on the agenda of the Recombinant Advisory Committee at its April 1978 meeting:

● To redefine the scope of the guidelines, including construction of a first list of "exempted exchangers";

● To review selected issues on the guidelines raised by the public commentators;

● To review the containment levels for experiments with viral DNA or viral vectors and with plant pathogens or viruses.

On the basis of the issues raised and the response of the Recombinant Advisory Committee, a decision and environmental impact assessment on proposed revisions is offered for public comment. The assessment that follows explains the present guidelines, the RAC's alternatives, the alternatives posed by the public and scientific commentators at the public hearing and in correspondence, the RAC's views on the issues raised at the April 1978 meeting, and the assessment of the revisions as proposed by the Director, NIH.

The Recombinant DNA Experimental Process

All living things, from subcellular particles to higher organisms, contain the specific information needed for their reproduction and functions. The basic source of this information is deoxyribonucleic acid (DNA), which is the principal substance of the genes—the units of heredity. Genes determine the characteristics of the species as well as individual traits such as size and eye color.

Each cell of an organism is composed of various organized structures, several of which contain DNA. Figure 1 illustrates a typical eukaryotic, or nucleated, cell. Bacterial cells (prokaryotic) are much less complex, showing fewer organelles and no organized nucleus.

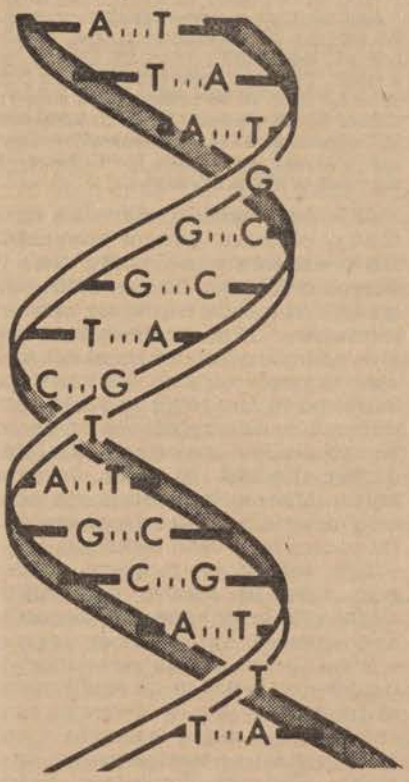
Figure 1

DNA plays to two roles: (1) provides information for the reproduction, growth, and functions of the cell, and (2) preserves and directs replication of this information and transfers it to the offspring. These two roles of DNA are common to animals, plants, single-cell organisms, and many viruses. The DNA of cells is mainly found in organized structures called "chromosomes."

Intracellular DNA also occurs outside of the chromosomes as separately replicating molecules. Such DNA molecules include the plasmids, found in bacteria; the DNA of chloroplasts, common to green plants; and the DNA of mitochondria, the energy-producing units of the cells of complex organisms. These DNA's, while not strictly part of the inherent genetic make-up of a cell, help define the cell's functional capability. Another type of DNA commonly found in cells is the DNA of infecting viruses.

During the past 30 years the structure of DNA molecules has been studied intensively, and it can now be described in much detail. The molecule may be compared to a long-twisted stepladder with thousands to millions of rungs. A short piece of DNA is represented in Figure 2.

Figure 2



The sides of the ladder are formed of sugar molecules (dexoyribose) attached end to end through phosphate groups. At right angles to each sugar molecule is one of four possible bases—adenine, guanine, thymine, and cytosine. The precise sequence of these bases, the rungs of the ladder, codes the information content. The "reading" of the code contained in the sequence of bases results in the formation of proteins, which in turn carry out most of the essential functions of the cell.

A gene is a portion of the DNA molecule which codes for the manufacture of a protein. In higher organisms, much of the DNA may not serve as genes in this sense, but may regulate the activity of nearby genes. It is possible to break open cells and isolate DNA, free of other cellular constituents.

The formation of "recombinant DNA" in the laboratory was made possible by a series of discoveries. W. Arber and D. Dussoix, in 1962, showed that bacteria contain substances called restriction enzymes. Serving to defend the bacteria against viruses, these enzymes can split foreign DNA molecules into specific fragments. R. Yoshimori, in H. Boyer's laboratory, isolated a restriction enzyme that was later found to split DNA into fragments whose ends stick together when they touch. In 1973 S. Cohen and others succeeded in combining genes of different species and introducing them into bacteria. Then they grew the bacteria in cultures, multiplying the combined characteristics.

The capabilities sketched here—to split DNA selectively, to recombine it by virtue of "sticky" ends, to reintroduce it into cells, and to cultivate the cells—constitute the recombinant DNA technique.

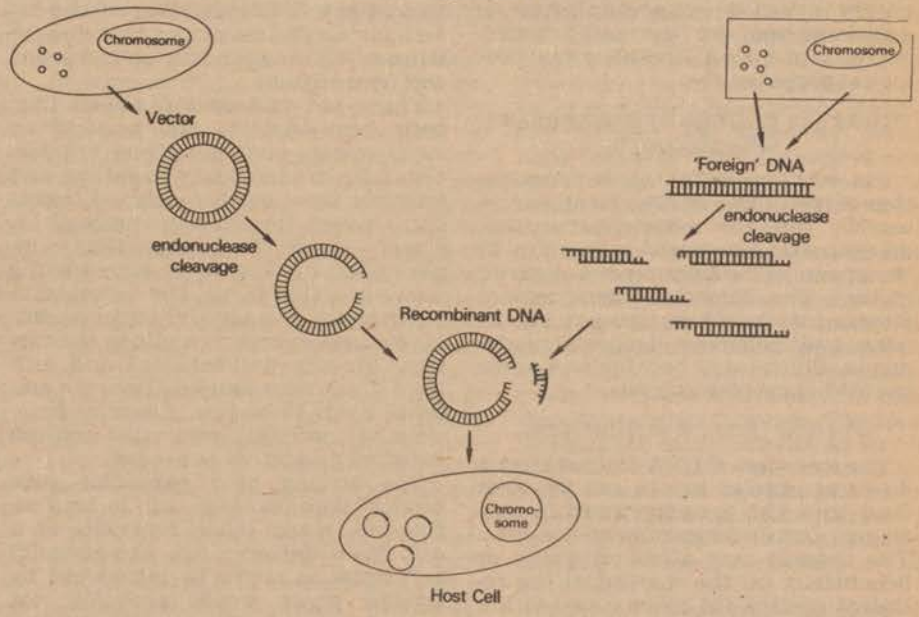
In the recombinant DNA experiments that are the subject of the NIH guidelines, the DNA can be derived from widely divergent sources. DNA from one of the sources may serve as a carrier, or "vector," for the insertion of the recombined DNA into a cell, or "host." The vector may be a plasmid, usually derived from the same species as the host, or it may be a virus. The DNA to be inserted is called the "foreign" DNA. When a large mixture of DNA fragments from the foreign source is used in the joining, the experiment is referred to as a "shotgun" experiment. In other instances, a particular DNA fragment of interest will be purified and then incorporated in the vector.

From a growth culture of the host cells, one containing the interesting DNA fragment is selected and allowed to multiply. The resulting population of identical cells is called a "clone." In some experiments the DNA will be extracted from the cells for study; in others, the properties of the cells themselves will be investigated.

In the experiments discussed in the guidelines, the host cells are generally single-cell microorganisms such as bacteria, or animal or plant cells that were originally obtained from living tissue but are grown as single cells under special laboratory conditions.

The process of producing recombinant DNA molecules and introducing them into cells is illustrated in figure 3.

Figure 3



The cell represented at the upper left contains chromosomal DNA and several separately replicating DNA molecules. The nonchromosomal DNA molecules can be isolated from the cell and manipulated to serve as vectors (carriers) for DNA from a foreign cell. Most DNA molecules used as vectors are circular. They can be cleaved, as shown, by enzymes (restriction endonucleases) to yield linear molecules with rejoinable ends.

At the upper right is another cell, represented here as a rectangle. It serves as the source of the foreign DNA to be inserted into the vector. This DNA can also be cleaved by enzymes. The rectangular cell could be

derived from any living species, and the foreign DNA might contain chromosomal or nonchromosomal DNA, or both.

In the next steps, the foreign DNA fragment is mixed and combined with the vector DNA, and the recombinant DNA is reinserted into a host cell. In most experiments this host cell will be of the same species as the source of the vector. The recipient cells are then placed under conditions where they grow and multiply by division. Each new cell will contain recombinant DNA.

Recombinant DNA technology represents a method that is applicable to many areas of biological research. Essentially, it represents a new tool. Investigations supported by many NIH Institutes and programs utilize this technique, much as a new instrument is applied in studying many different things. Areas of biological research to which recombinant DNA experiments are underway include the study of bacterial enzymes and metabolism, the synthesis of hormones, the reproduction of viruses, the organization of chromosomes, and the structure and regulation of genes. Except for studies to improve the technology, NIH sponsors no program on recombinant DNA as such; but recombinant DNA technology is used, where applicable, as an additional tool for increasing understanding of normal and abnormal biological processes.

Risks and Benefits of Recombinant DNA Research

Research, by definition, is investigation of the unknown. The results of research, whether beneficial, neutral, detrimental, or some combination of these, cannot be fully predicted ahead of time. The following discussions are assessments based on present knowledge and collective technical judgments. Unexpected benefits and unexpected hazards are possible.

Possible Hazardous Situations

The insertion of DNA derived from a different species into a cell or virus (and thus the progeny thereof) may change certain properties of the host. The changes may affect adversely or beneficially (a) the survival of the recipient species, (b) other forms of life that come in contact with the recipient, and (c) aspects of the nonliving environment. Current knowledge does not permit accurate assessment of such effects in contemplation of every recombinant DNA experiment. At present it is only possible to speculate on ways in which the presence of recombinant DNA in a cell or virus could bring about these effects.

It should be noted that there is no known instance in which a hazardous agent has been created by recombinant DNA technology. The following discussion is speculative and considers ways in which hazardous agents might be produced. In principle, the analysis is applicable to animals, including humans, and to plants, when potential effects on complex organisms are described.

The effect of foreign DNA on the survival of recipient species (host cell, plasmids, or viruses)

The effect of foreign DNA on the survival of receipent species is important to the discussion of possible hazards of recombinant DNA experiments. A recipient species may acquire a potential for harmful effects as a result of the foreign DNA, but the possibility of the occurrence of the harmful effects will depend on the survival of the recipient and its ability to multiply. If acquisition of foreign DNA increases the probability of survival and multiplication, the possibility of harmful effects will increase. Similarly, if acquisition of foreign DNA decreases the probability of survival or multiplication, the possibility of harmful effects will decrease. It is important to recognize, in evaluating the potential for harmful effects, that significant infections of animals and plants by bacteria or viruses may require contact with a critical number of the infectious agents, quantities that may be large or small depending on the agent and the recipient.

There are various indications that both host bacteria and plasmid or virus vectors containing inserted foreign DNA are less likely to survive and multiply than are the original organisms, except for the very unusual instances where the foreign DNA supplies some function, such as antibiotic resistance, that favors the organism in a particular, non-natural environment. (*1*) Natural selection results in the survival of only well-balanced and efficient organisms; unneeded genetic material tends to be lost. Essential functions are carefully controlled and are switched on and off as needed.

The activity of a particular gene product depends upon, and in turn influences, many other functions of a cell. Such uncontrolled, non-essential properties as might be introduced by foreign genes would probably not result in any advantage to the survival and multiplication of an otherwise well-balanced organism. Rather, the new properties might be expected to confer some relative disability. It is unlikely that elimination of a gene product by insertion of a foreign DNA sequence would be advantageous. More likely than not, any new properties derived from insertion of foreign DNA would confer some relative disability on the recipient organism. Therefore, it is probable that bacterial cells, plasmids, or viruses containing inserted foreign DNA would multiply more slowly in nature than the same cells or vectors without foreign DNA; and in a natural competitive environment, those organisms containing recombinant DNA would generally be expected to disappear. For bacterial hosts, the rate of disappearance would depend on the relative rate of growth compared to other, competing bacteria. The following calculation demonstrates this point.

Assume that a new organism constitutes 90 percent of a population, but grows 10 percent less rapidly than its natural counterpart. The new organism will drop from a concentration of 90 percent to a concentration of 0.0001 percent (1 part in 1,000,000) in 207 generations. If the generation time of the natural organism is 1 hour, this amounts to about 8½ days.

Although unlikely, there is a chance that a bacterial host of recombinant DNA will grow more rapidly than if it were lacking the foreign DNA, especially if the cells encounter new environments. (The calculation given above can be applied here also.) A relevant example of such a situation can be found in the rapid and widespread increase in the resistance of bacteria to clinically important antibiotics during the last 20 years. It is well known that such resistance is genetically determined, and genes specifying resistance have been described. (*2*)

The ability of recipient bacterial host cells to survive and multiply might also be enhanced by acquisition and expression of a foreign gene conferring the ability to metabolize particular nutrients. In an environmental niche containing the nutrient, such a recombinant might compete successfully against organisms native to the niche. Thus an important nutrient there might be destroyed. Also, if the native organisms were performing beneficial functions, those functions could be lost upon the successful establishment of the recombinant in the niche.

These examples serve to illustrate some of the complexities involved in determining whether the insertion of a given fragment of foreign DNA will be advantageous or disadvantageous to the recipient organism: The nature of the inserted genes, the nature of the environment, and the relation between the two must be considered. However, this analysis is necessarily simplistic. In the absence of the highly specific relationships that are, for example, apparent in the case of antibiotic resistance, very little is understood about how the totality of the genetic makeup of a given organism or species contributes to its competitive advantage even in a defined ecological niche. Modern evolutionary theory does not provide useful frameworks for analy-

sis. There are in fact current major controversies concerning the role of natural mutations in evolution, and the same questions are relevant to the issues raised by recombinant DNA research.

Because potentially useful vectors such as plasmids and viruses may be transferred from the initial recipient host cell to other cells, independent of the growth and survival of the host, it is also necessary to consider survival of the vectors. Plasmids and viruses occur widely in nature. Any particular plasmid or virus will normally multiply only within a limited number of species. Thus, for example, viruses that infect particular bacteria neither multiply nor cause disease in the cells of other bacterial species or complex organisms. In many instances, they do not even enter the cells of any organism other than the particular natural host.

Only limited information concerning the effect of foreign DNA insertions on the survival or transferability of plasmid and viral vectors is available. In the case of plasmids, the factors contributing to their maintenance or loss from cells in natural environments, even without insertion of a foreign DNA, are not clearly understood. (*2*) One exception is the selective advantage for maintenance provided by an antibiotic-resistance gene on the plasmid.

Also, some plasmids are known to confer on host cells the ability to manufacture substances poisonous to closely related cells, thus giving the poison-producers special advantage in a competitive situation. Insertion of a foreign DNA fragment into the DNA sequence coding for the poison has been shown to eliminate production of the poison, (*3*) decreasing the likelihood that the cells and their resident recombinant DNA will survive in nature.

Experiments carried out during the last few years have yielded only minimal information on the stability of plasmids containing foreign DNA in host cells, or on the stability of the foreign fragment itself. For experimental purposes, cells containing recombined plasmids are generally grown under conditions especially designed to increase the stability of the plasmid (called "selective" conditions.) For consideration of the loss of the plasmids in natural environments—the important point for matters of safety—the stability of the plasmid or recombined DNA under ordinary, or nonselective, conditions needs to be known. A review of a limited number of unpublished observations indicates that generalizations as to the rate of loss of the recombined plasmid relative to the original are impossible.

The ability of a plasmid to be transferred from the original laboratory host to another cell and thereby perpetuate itself is also important. In short, certain plasmids are incapable of being transferred except under particular and inferquent conditions. Others transfer more readily. Since the ability to be transferred depends on multiple factors, (*2*) it is not likely to be increased by insertions of a single foreign DNA fragment. Other than this, no generalization concerning the effect of a foreign DNA fragment on transferability can be made.

The effect of bacteria and veruses containing recombined DNA on other forms of life

The analysis leading to the guidelines centered on the possibility of deleterious effects, since the concern was the health and safety of living organisms, including humans, and the environment. Agents constructed by recombinant DNA technology could prove hazardous to other forms of life by becoming pathogenic (disease-producing) or toxigenic (toxin-producing), or by becoming more pathogenic or toxigenic than the original agent.

There are two basic mechanisms by which a recipient micro-organism might be altered with regard to its pathogenicity or toxicity as a result of a resident recombinant:

(1) *The recombinant DNA may result in formation of a protein that has undesirable effects.* The case in which bacterial cells are used as carriers of foreign DNA is discussed first. A foreign protein, specified by the foreign DNA, might act after being liberated from the micro-organism, or it could function within the micro-organism and alter, secondarily, normal microbial cell function in such a way that the cell is rendered harmful to other living things. Either means depends on the expression of the foreign genes; that is, the information in the foreign genes must be used by the recipient bacterium to produce a foreign protein. Examples of proteins that might prove harmful to other organisms are hormones, enzymes, and toxins.

Present evidence suggests that foreign DNA from bacteria of one species, when inserted into bacteria of another species, may be expressed in the recipient, depending on the similarities of the protein synthesis mechanisms in the two organisms. (*4*) For example, if the donor of the foreign DNA produces a toxic substance, than the recipient cell may produce such a substance, provided the gene for the toxic substance is present in the recombinant. The recipient may or may not be more hazardous than the original donor organism, depending on the relative ability of the two organisms to grow and infect an animal or plant species at risk.

Expression of foreign genes from complex organisms (yeast, fruit flies) in cloned bacteria has recently been demonstrated experimentally. (*5*) In other experiments, insertion of a synthetic gene into *E. coli* led to the production of somatostatin, a hormone found in the mammalian brain. (*6*)

Analogous issues must be considered for the case in which animal viruses are the carriers of foreign DNA. Inadvertent dispersal of such viruses outside the laboratory might result in entry of the recombinant DNA into cells of living organisms. The foreign genes might be expressed, resulting in the uncontrolled synthesis of a normal protein or the formation of a protein foreign to the infected cell. Currently, few relevent experimental data are available. (*3*)

(2) *The recombined DNA may itself cause pathogenic or toxic effects.* As discussed above, foreign DNA inserted in a bacterial gene might so alter the microbial cell's properties that it becomes harmful to other organisms. It is also necessary to consider situations in which DNA molecules themselves may escape from the laboratory or from the experimental host cell an enter cells of living organisms with which they come in contact. Free DNA molecules are themselves relatively fragile, and the probability that they would survive, in a significant form or for a significant time, in air, water, or any medium, is considered remote. DNA that is protected in any of a variety of ways within cells and viruses might be released either into, or close to, a living cell.

When a cell or virus dies, or comes close to or invades the tissue of another living organism, the recombinant DNA may effectively enter a new cell. A hazardous situation similar to that described above might ensue if foreign proteins were manufactured in this "secondary" recipient. The recombinant DNA might survive as an independent cellular component, or it could recombine by natural process with the DNA of the secondary recipient. Various possible deleterious consequences of such a recombination may be considered.

If the secondary recipient is another micro-organism, the considerations described earlier apply. If the secondary recipient is one of the cells of an animal or plant, the possible effects are different. They include alterations of normal cellular control mechanisms, synthesis of a foreign protein (such as a hormone), and insertion of genes involved in cancer production (if, for example, the foreign DNA were derived from a cancer-producing virus).

It should be pointed out that the likelihood of such a mechanism causing inheritable changes in the offspring of complex organisms is extremely low because of the protection afforded germ-line cells (eggs and sperm) by their location. Thus, it is highly improbable or perhaps impossible for recombined foreign DNA to reach germline cells at a time in their life when secondary recombination can occur. With one-celled organisms, plants, or simple multicellular organisms, the probability of heritable change resulting from secondary recombination is higher.

What is the probability of secondary recombination between prokaryotes and eukaryotes in nature? It is generally held that the recombination in nature is more likely if similar or identical sequences of bases (rungs in the DNA ladder) occur in the two recombining DNA's.(*7*) The greater the degree of similar sequences, the more likely is recombination. In general, the more closely two species are related, the more likely it is that similar sequences will be found in their DNA's. Thus, DNA from primates has more DNA sequences in common with human DNA than does DNA from mice, or fish, or plants. Recombination may also occur between DNA's not sharing sequences, but at lower frequencies.

It is possible that the capacity for interspecies recombination between distantly related species exists in nature. For example, bacteria in animal intestines are constantly exposed to fragments of animal DNA released from dead intestinal cells. Significant recombination, however, would require the uptake of intact segments of animal DNA and their subsequent incorporation into the bacterial DNA. Such uptake and incorporation has been demonstrated experimentally. The frequency of such events in nature is unknown.

Similarly, there are very few data permitting assessment of the reverse process; namely, the incorporation of bacterial DNA into the cells, or DNA, of more complex organisms. Although there are reports of experiments in which bacterial DNA was inserted into animal and plant species and production of the bacterial protein followed, the process is very inefficient and many investigators have been unable to repeat these experiments.(*8–10*)

There are certain well-documented instances in which the DNA's of different living things become more or less permanently recombined in nature. These instances involve recombination between the DNA's of nonchromosomal genes, such as those of viruses or plasmids, or between the DNA's of viruses or plasmids and chromosomal genes. The former instance, for example, is the mechanism behind the rapid spread of resistance to antibiotics among different bacterial species.(*2, 11*) This spread accompanied the prevalent use of antibiotics in medicine and agriculture. Another example is the insertion of DNA from the bacterium *Agrobacterium tumefaciens* into plant cells.(*13*)

Expected Benefits of Recombinant DNA Research

Benefits may be divided into two broad categories: an increased understanding of basic biological processes, and practical applications for medicine, agriculture, and industry.

At this time, most of the practical applications are speculative. It is important to stress that the most significant results of this work, as with any truly innovative endeavor, are likely to arise in unexpected ways and will almost certainly not follow a predictable path.

Increased understanding of basic biological processes

There are many important fundamental biomedical questions that can be answered or approached by DNA recombinant research. In order to advance against inheritable diseases, we need to understand the structure of genes and how they work. The DNA recombinant methodology provides a simple and inexpensive way to prepare large quantities of specific genetic information in pure form. This should permit elucidation of the organization and function of the genetic information in higher organisms. For example, current estimates of the fraction of this information that codes for proteins are simply educated guesses. There are almost no clues about the function of the portions of DNA that do not code for proteins, although these DNA sequences are suspected of being involved in the regulation of gene expression.

The existing state of ignorance is largely attributable to our previous inability to isolate discrete segments of the DNA in a form that permits detailed molecular analysis. Recombinant DNA methodology removes this barrier. Furthermore, ancillary techniques have been developed whereby pure DNA segments that contain particular sequences of interest can be identified and selected. Of particular interest is the isolation of pure DNA segments that contain the genes for the variable and constant portions of the immunoglobin proteins. The analyses of such segments obtained from both germ-line and somatic cells should be of inestimable value in determining the mechanism of immunologic diversity.

A major problem in understanding the mechanism by which certain viruses cause cancer is how and where the infecting or endogenous viral genomes are integrated into the cell's chromosome.(*12*) This bears on the question of how the integrated viral genes affect cellular regulation, thus leading to the abnormal growth characteristics of cancer cells. With the recombinant DNA techniques for isolation and purification of specific genes, this research problem is reduced to manageable proportions. It is possible to isolate the desired DNA segment in pure form. Large quantities can be obtained for detailed study by simply extracting a culture of the bacteria carrying the viral DNA segment in a plasmid.

Important recent achievements in recombinant DNA research

It was anticipated (see EIS of October 1977) that the ability to excise, isolate, and amplify specific segments of DNA from higher organisms would provide an unprecedented opportunity to study the structure of eukaryotic genomes and to correlate the results with concepts of how they evolved and are regulated. Recent work has yielded much more. Indeed, some of the genomic structures discovered through use of recombinant DNA techniques have occasioned a substantial reassessment of several major paradigms of molecular biology.

Many of the initial studies using recombinant DNA techniques focused on DNA sequences that are repeated in the genomes of eukaryotes. In some instances, these repeated sequences specify an RNA product, such as ribosomal RNA, or fulfill a function as yet unknown—for example, the sequences called "satellite" DNA. The organization of such sequences is being examined extensively with recombinant DNA techniques(*14–20*).

The genes that specify ribosomal RNA are repeated in the eukaryotic genome several hundredfold. It has been known for some years that in a variety of species, such as the frog *Xenopus laevis*, these genes are arranged as a series of tandem repeats. Each set of ribosomal genes is separated from its neighbors by regions of DNA, of varying length, that are not transcribed. Cloning of several of these nontranscribed, or "spacer," regions has allowed analysis of the manner in which they are related to one another(*21, 22*) and proposals of evolutionary mechanisms by which they may have arisen.

Moreover, the availability of these cloned DNA sequences has made it possible(*23*) to localize the DNA site at which the transcription of the ribosomal genes is initiated. The exact DNA sequence at this site is being determined. Such information will further the understanding of the mechanisms

that regulate gene expression and the construction of new host/vector cloning systems.

Many of the basic concepts of molecular biology have had to be based upon work on prokaryotes. These concepts have influenced the interpretation of data on the structure and possible mode of functioning of the eukaryotic genome. Recombinant DNA technology, however, has allowed the structure of the genomes of higher organisms to be examined in a manner previously restricted to studies on bacteria. Some of the results have upset earlier assumptions. For example, the cloning of eukaryotic DNA sequences that specify the proteins β globin,(*24, 25, 30, 34*) ovalbumin,(*26, 27, 28, 32, 33*) and immunoglobulin(*29, 31*) has demonstrated that certain basic facts of DNA organization in prokaryotes are not applicable to eukaryotes. The major new finding of "intervening" sequences in these higher forms (discussed in footnote 13 to the "Introduction and Overview" of the accompanying decision document) may provide an explanation for the cause of the hereditary disorder, β^+ thalassemia. It also demands a reconsideration of the basic mechanisms of differentiation and evolution.

The work on immunoglobulin(*29, 31*) has shown that the DNA sequences that encode two different regions of an immunoglobulin polypeptide are widely separated in germ-line cells. During differentiation, these DNA sequences move closer together but do not become contiguous. Detailed examination of DNA fragments has resolved one of the fundamental and longstanding puzzles of immunology. Very recent data indicate that the intervening sequences found in the gene for ovalbumin differ from individual to individual(*32, 33*), indicating that genetic variability may occur within a species to an extent not previously imagined.

Practical implications

The possibility that recombinant DNA techniques could be used to alter the properties of recipient organisms, or to produce desired substances, such as peptide hormones, rests largely upon two factors: (1) the fidelity of the cloning procedures and (2) the ability to obtain expression of the cloned DNA sequence. It has been demonstrated that cloning does provide faithful copies of the original sequence.(*34*) More recently, experiments in which cloned fragments of yeast DNA were introduced into *E. coli* have provided further evidence that fidelity is maintained and that expression of cloned sequences can be achieved. All the bacterial strains used contained genetic lesions that rendered them incapable of synthesizing a particular amino acid. However, the yeast DNA sequences were capable of correcting these deficiencies(*35*) and were shown to specify the synthesis of a yeast protein that substituted successfully for its defective bacterial counterpart.

There have been several accomplishments during the last year and a half that may be expected to yield both economic and medical benefits in the near future. Work has begun on the cloning of the nitrogen fixation genes of the bacterium *Klebsiella pneumoniae.*(*36*) The introduction of these genes into appropriate plant or bacterial hosts may drastically reduce the requirement for nitrogenous fertilizers. Such fertilizers, currently consumed at the rate of 40 million tons per year, are synthesized by processes involving large energy costs.

An area that has proved to be extremely productive has been the cloning of DNA sequences specifying peptide hormones. During 1977 there were succeses in the cloning of genes for the following hormones:

Insulin.(*37*)

Somatostatin,(*6*) a hormone that inhibits the secretion of glucagon, insulin, and growth hormone; potentially useful in the treatment of acromegaly, acute pancreatitis, and insulin-dependent diabetes.

Growth hormone,(*38*) used in the treatment of dwarfism; in short supply throughout the world.

Somatomammotropin,(*39*) a hormone secreted by the placenta; appears to act on maternal metabolism to insure that the fetus obtains nutrients required for normal growth.

The cloning of the somatostatin gene is particularly noteworthy, since the gene was synthesized chemically, thus avoiding any risk of inadvertant cloning of contaiminating sequences, and was then inserted into a specially constructed plasmid vector. The inserted DNA sequence was expressed and an inactive precursor of somatostatin was synthesized and isolated. From this, active somatostatin was subsequently liberated. Because the polypeptide precursor synthesized within the *E. coli* K-12 host is inactive, the procedure also decreases markedly and likelihood that the host cell itself could be hazardous. This same strategy may be used in the cloning of any of the small peptide hormones.

Long-range implications

The experimental situations treated in the guidelines are those that appear feasible either currently or in the near future. The experiments primarily involve insertion of recombined DNA into bacteria or into single cells derived from more complex organisms and maintained under special laboratory conditions. It is only in the case of plants that the guidelines cover experiments involving insertion of DNA into cells capable of developing into complex, multicellular organisms. The guidelines and the discussions leading to their development have focused on problems of safety.

It is possible that techniques similar to or derived from current recombinant DNA methodology may, in the future, be applicable to the deliberate modification of complex animals, including humans. Such modification might serve to correct an inherited defect in an individual, or to alter heritable characteristics in the offspring of individuals or a given species. The latter type of alteration has been successfully achieved in agriculture for centuries by classical breeding techniques. It may be that recombinant DNA methods, should they develop in appropriate ways, will offer new opportunities for specificity and accuracy in animal breeding. It should be noted, however, that the techniques covered by the NIH guidelines involve the recombining of DNA fragments in the test tube, and the guidelines prohibit the deliberate release into the environment of any organism containing recombinant DNA molecules.

Should the deliberate application of such methods for the correction of individuals genetic defects or the alteration of heritable characteristics in man ever become possible, it will raise complex and difficult problems. In addition to philosophical, moral, and ethical questions of concern to individuals, serious societal issues will be involved. Broad discussion of these problems in a variety of forums will then be required to inform both private and public decisionmaking.

Possible Deliberate Misuse

In the event that recombinant DNA technology can yield hazardous agents, such agents might be considered for deliberate perpetration of harm to animals (including humans), plants or the environment. The possibilities include biological warfare or sabotage. Because it is not known whether recombinant DNA technology can yield such agents, discussion of these problems, such as theft by saboteurs, is hypothetical and difficult. With regard to biological warfare, the use of recombinant DNA for such purposes is prohibited by the Biological Weapons Convention. In a statement to the Conference of the Committee of Disarmament, on August 17, 1976, Ambassador Joseph Martin, Jr., made the following remarks on the subject:

> When advances in science and technology are made, it is natural to ask about their possible use for hostile purposes and whether or not such uses are prohibited or restricted by existing international agreements. In the case of potential use of recombinant DNA molecules for weapons pur

poses, it is our view that such use clearly falls within the scope of the Convention's prohibition.

This interpretation is based upon the negotiating history as well as the explicit language of the Convention, and we believe that it is shared by the other signatories. I do not believe it is possible to read the Biological Weapons Convention and come to any other conclusion. According to the Preamble, the States Parties are "determined, for the sake of all mankind, to exclude completely the possibility of bacteriological (biological) agents and toxins being used as weapons." The intent of Article I, which begins, "Each State Party to this Convention undertakes never in any circumstances * * *," is equally forceful and clear. To take a more restricted view would rob the Convention of much of its value and could even lead to States to call into question its scope and continued viability. These were the views of the United States when the Convention was negotiated and ratified. They are still its views today. This is a matter of great importance to my Government and one on which doubt cannot be permitted to exist.

It is noteworthy that, prior to his statement, Dr. David Baltimore had requested an opinion from James L. Malone, General Counsel of the United States Arms Control and Disarmament Agency, on whether the Biological Weapons Convention prohibits production of recombinant DNA molecules for purposes of constructing biological weapons. On July 3, 1975, Mr. Malone replied: "In our opinion the answer is in the affirmative. The use of recombinant DNA molecules for such purposes clearly falls within the scope of the Convention's provisions."

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I. Scope of the Guidelines

Analysis of Current Guidelines [1]

For the purposes of the current Guidelines, recombinant DNA experiments are defined as those involving molecules that consist of segments of DNA from different sources which have been joined together in cell-free systems, and which have the capacity to replicate in some host cell, either autonomously or as an integrated part of the host's genome. The host cells in these experiments are generally single living cells. They may be microorganisms such as bacteria, or animal or plant cells grown in tissue culture.

General principles

The Guidelines start with a statement of general principles, which are consistent with the conclusions in the report of the International Conference on Recombinant DNA Molecules held at Asilomar, Calif. in February 1975. The first principle is that certain experiments may be judged, in the light of currently available information, to present such serious potential hazards that they should not be attempted at this time. Second, a large group of feasible experiments appear to pose less or no potential hazard, and can therefore be performed under appropriate safeguards if the information or benefits sought cannot be obtained by conventional methods. Third, the more serious the nature of any presumed consequence, the more stringent should be the safeguards against escape of the potentially hazardous agents.

Experiments to be deferred

The first class of experiments described in the Guidelines are those that are not to be carried out at the present time. While it may be argued that a combination of maximal physical and biological safeguards could essentially contain these recombinants, the magnitude of the possible dangers if containment were to fail dictates that these experiments be deferred. This class of experiments includes the following:

- Those in which any of the recombinant DNA derives from pathogenic organisms listed under classes 3, 4, and 5 of the document *Classification of Etiologic Agents on the Basis of Hazard,* published by the Center for Disease Control (CDC) of the U.S. Public Health Service, or from oncogenic (cancer-causing) viruses classified by the National Cancer Institute as "moderate risk." The CDC document categorizes naturally occurring organisms and viruses known to be pathogenic to man and agriculturally important species, on a scale of increasing hazard from 1 to 5.
- Those characterized by deliberate formation of recombinants containing the genes for potent toxins. Examples are botulinus or diphtheria toxins, and venoms from insects and snakes.
- Those involving deliberate creation from plant pathogens of recombinant DNA's that are likely to increase the virulence of the pathogenic material or the range of susceptible species.
- Those involving transfer of a drug-resistance trait to microorganisms that are not known to acquire it naturally if this could compromise the use of a drug to control disease in humans, animals, or plants.

Other restrictions concern the volume of recombinant DNA to be produced and the deliberate release of organisms into the environment:

- Experiments with recombinant DNA's known to make harmful products must be limited in scale to quantities of fluid less than 10 liters. The RAC may make exceptions for particular experiments deemed to be of direct societal benefit, if appropriate equipment is used.
- The creation of organisms with ability to carry out useful environmental functions has been contemplated. Release of such organisms into the environment may be required to test their efficacy, and certainly to make use of them. The Guidelines, however, prohibit at present the release of any organism containing a recombinant DNA molecule.

Alternatives: RAC-Proposed Revisions

It was the determination of the Recombinant Advisory Committee that advances in knowledge pertaining to recombinant activities in past years warranted significant revisions in the purpose, definition, and prohibition sections of the NIH Guidelines. A comparison of the "purpose" language of the current Guidelines with that of the proposed revised guidelines of the RAC (hereafter called "PRG-RAC") reveals that the standards in the latter set are meant to pertain to recombinant DNA molecules *in organisms.* The analogous language in the current Guidelines addresses recombinant DNA molecules whether or not they are contained within a cell or virus. The rationale for this change is that DNA by itself (commonly referred to as "naked" DNA) is extremely unlikely to be hazardous under experimental conditions, as it is rapidly inactivated in nature.

The definition in the PRG-RAC consists of two parts: (1) an operational definition of recombinant DNA and (2) a qualification that the Guidelines would pertain only to "novel" recombinant DNA's. The operational definition does not differ significantly from that in the original Guidelines.

The second part, however, calls for the creation of a list of organisms that exchange genetic information in nature, commonly referred to as "non-novel exchangers." Recombinant DNA formed with DNA from such organisms would be exempted from the provisions of the Guidelines, with the rationale that there is no justification for requiring stringent containment procedures for the handling of recombinations that occur regularly in nature and are not known to be associated with any special hazards.

The provision of an open-ended listing was recommended rather than issuance of a blanket exemption, because this would allow the RAC and NIH to consider evidence that (1) the putative gene transfers do take place naturally and (2) that their exemption from the Guidelines is justifiable.

Although the PRG-RAC deal with prohibited experiments under part III, this assessment, for purposes that become apparent below, will consider the definition, exemptions, and prohibitions together under part I.

A major proposed revision would give the Director, NIH, authority to grant exceptions to any of the six prohibitions. Such a determination must be based upon the recommendation of the RAC, and weight must be given in the decision-making "both to scientific and societal benefits and to potential risks." The rationale for this proposed change was the desire of the RAC not to bar automatically the conduct of experiments desirable for some compelling social or scientific reasons—for example, risk assessment.

Alternatives: Public Commentators

There was considerable discussion at the public hearings over the scope of

[1] As published in the Federal Register, July 7, 1976 (41 FR 27902).

the Guidelines. Some mentioned that the Guidelines were too narrow in their preocuppation with recombinant DNA, as there are other forms of genetic research capable of producing cells and organisms of unknown potential hazard. It was further suggested that the title of the Guidelines be modified to reflect their concern with experiments involving prokaryotes and cells in culture and that a companion document be released dealing with higher eukaryotes. On the other hand, it was also argued that genetic research has not received attention far beyond its due and that other matters of experimentation await their turn.

It is true that other techniques in genetic research, such as cell fusion and chromosome transfer, may result in formation of recombinant molecules. However, there are inherent in these techniques a range of natural barriers to the formation of hazardous organisms which apparently afford adequate containment, making unnecessary the issuance of Federal standards. Such techniques have been used in the laboratory for decades with no known harmful effects on either the public health or the environmnent. The entire area of laboratory safety is of primary concern to NIH and is the subject of constant review and attention.

There were several suggestions that the purpose of the Guidelines be more clearly stated and that terms be more precisely identified. Therefore, the sections "Purpose," "General Applicability," and "General Definitions" have been added to part I of the guidelines now being proposed by NIH—hereafter called "PRG-NIH."

Purpose of the guidelines

The Introduction to the 1976 Guidelines states that "the purpose of these Guidelines is to recommend safeguards for research on *recombinant DNA molecules.*" As noted above, to eliminate the handling of naked DNA from the Guidelines, the PRG-RAC proposed this passage to read that the purpose is to "establish procedures for handling *organisms and viruses containing recombinant DNA molecules.*"

This proposed revision would have had the effect of removing from coverage by the guidelines certain experiments that are now prohibited by the 1976 Guidelines—for example, deliberate formation of "naked" recombinant DNA-containing genes for the biosynthesis of potent toxins. The Director, NIH, proposes to resolve this issue conservatively. The language in the PRG-NIH, therefore, clearly states that the Guidelines are intended to pertain to the construction and handling of naked recombinant DNA molecules as well as organisms and viruses containing such molecules.

General applicability

Many commentators urged that a statement of general applicability of the Guidelines be included in an early part. The issues relate to (1) the applicability of the Guidelines to non-NIH-funded research with recombinant DNA at institutions receiving NIH funds for this purpose, (2) the applicability of the Guidelines to NIH-supported recombinant DNA research conducted in foreign countries, and (3) the location of responsibility for ensuring compliance with the Guidelines. Therefore, a section entitled "General Applicability" now appears after the "Purpose" section in Part I of the PRG-NIH.

The existence of guidelines for recombinant DNA research assumes their general application. Partial adherence within an institution would defeat the purpose of extending maximal protection to the community. Thus, it would be inconsistent for NIH to provide funds for biomedical research activities to an institution that did not meet the standards of the Guidelines in all of its recombinant DNA research, regardless of the source of funding. This principle is now stated explicitly in the Guidelines, and we intend to consider withholding NIH funds as a sanction against violation.

Rules must be established for the conduct of recombinant DNA activities funded by NIH in other countries. Generally, the requirements in force in those countries shall apply. A Memorandum of Understanding and Agreement (MUA) must still be filed with NIH, indicating specifically which guidelines will govern the activities; and NIH reserves the right to withhold funding if the safety practices to be employed are not reasonably consistent with the NIH Guidelines. An explicit statement about this has been inserted in the PRG-NIH.

Part IV of the PRG-NIH defines the responsibilities of all individuals and organizational entities involved in the conduct and review of a recombinant DNA activity. Two years of experience with administering the NIH Guidelines have demonstrated that the ultimate responsibility for ensuring compliance must be borne by the institution where the research is being done. This implies some discretion under well-defined limits for interpretation of common standards, and imposes a requirement for local expertise other than the investigator's. Accordingly, part I of the PRG-NIH now requires that an individual receiving NIH support for recombinant DNA research be associated with an institution that is willing and able to accept the responsibilities and conditions of local governance, described more explicitly in Part IV of the PRG-NIH.

Definition of recombinant DNA molecules

It became apparent from the comments received that the PRG-RAC definition was inadequate in not addressing the handling of recombinant DNA molecules containing segments of chemically synthesized DNA. It was decided that the most effective way to achieve this objective is simply to include "natural or synthetic DNA" in the definition of a recombinant DNA molecule, and this has been inserted in the PRG-NIH definition. A new section, therefore, has also been aded to part III of the PRG-NIH giving containment levels for work with recombinant DNA molecules containing synthetic DNA.

A perceived ambiguity in the PRG-RAC definition has been corrected by the inclusion of language within the PRG-NIH definition which explicitly states that DNA molecules resulting from the replication of recombinant DNA molecules are subject to the safety provisions of the Guidelines.

Finally, no other provisions of the PRG-RAC definition evoked as much comment as did the wording to exclude "non-novel" recombinant DNA from the standards. The ambiguity of such phrases as "known to exchange chromosomal DNA" and "by natural physiological processes" was strongly noted. A greater degree of clarity and objectivity is needed. Thus, it has been decided to eliminate in the PRG-NIH the two conditions cited above as criteria for exemption from the Guidelines. Staff discussions of the public comments made it clear that inclusion of exemption provisions within the definition itself was not desirable; several attempts at appropriate language did not bear careful scrutiny.

Given this situation, and also the realization that certain categories of recombinant DNA experiments are indeed so apparently free of causing harm that they should not come under the Guidelines, the criterion of "novelty" was removed from the definition and used as a basis for the development of a new section entitled "Exemptions."

Exemptions

The nature of the public comments on the PRG-RAC exclusion of non-novel exchangers can be divided into two categories—those that pertain to the proposed standards and those to the proposed process.

The standards proposed by the PRG-RAC were that novel recombinant DNA's consist of "segments of any DNA from different species not known to exchange chromosomal DNA by natural physiological processes * * *. In general, recombinant DNA molecules * * * will not be considered novel when all the components are derived

from genomes known to replicate within the organism used to propagate the recombinant DNA." This is qualified, however, by a footnote stating that "recombinant DNA formed between segments of eukaryotic viral DNA and any eukaryotic DNA * * * shall not be excluded * * * until such time as there is more information about the extent of naturally occurring recombinational events between these DNA's."

The public comments on these standards raised a number of issues. For example, some said that safety rather than novelty should be the criterion for exclusion. That is, any recombinant molecule that poses a threat to the public health or the environment should be covered by the Guidelines regardless of whether the molecule is a novel one. Others held that the proper criterion should not be safety, but rather whether the potential hazard of the recombinant DNA molecule differs significantly in degree or in kind from those found in nature or from biohazards that are successfully handled by conventional methods. It proved impossible to reconcile these differences of opinion in the deinition itself and so an "Exemptions" section was drafted by NIH staff in conjunction with an RAC working group. It should be noted that no provision in that section may be cited to exempt from the Guidelines an activity listed in the "Prohibitions" section.

The first exemption concerns recombinant DNA molecules that are not in organisms or viruses. This is in recognition that "naked" DNA, which is rapidly inactivated in nature, is extremely unlikely to be hazardous under experimental conditions. To guard, however, against the remote possibility that potentially harmful naked recombinant DNA will be incorporated into an organism, the handling of certain naked recombinant DNA molecules described in the "Prohibitions" section remains prohibited. It should also be noted that the concept of extremely low hazard of naked recombinant DNA was included in the PRG-RAC in the section on "Handling Recombinant DNA Molecules" at the end of Part III. This language is more appropriately presented under the "Exemptions" section.

The section exemption pertains to recombinant DNA molecules consisting entirely of DNA segments from a single non-chromosomal or viral source. This statement clarifies a category of "self-cloning" experiments that are considered safe enough to be excluded from the Guidelines. This is a concept which the RAC tried to convey in the PRG-RAC definition by use of the phrase "different genomes" but which some commentators found ambiguous.

The third exemption concerns "self-cloning." It exempts from the Guidelines recombinant DNA molecules made entirely from the DNA of a single organism, including the indigenous plasmids, viruses, mitochondria, or chloroplasts, when propagated only in that oranism (or a closely related strain of the same species). This partially responds to the suggestion made by many commentators that experiments previously classified as P1+ EK1 be excluded from the Guidelines. It also covers some of the cases the RAC was including in the concept of "novelty" and "different genomes." This exemption, however, does not include recombinant DNA molecules formed between viral DNA and eukaryotic host DNA. In this regard it is analogous to footnote 1 of the PRG-RAC.

The fourth exemption covers "certain specified recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes." In this case a list is prepared and periodically revised by the Director, NIH, on the recommendation of the RAC, after appropriate notice and opportunity for public comment. This list is analogous to the list of "non-novel exchangers" proposed in the PRG-RAC. Appendix A to the PRG-NIH gives a proposed list for this exemption. This list is discussed in Appendix D to the present document.

The fifth exemption allows the Director, NIH, on the recommendation of the RAC and after appropriate notice and opportunity for public comment, to exempt other classes or recombinant DNA molecules if he finds that "they do not present a significant risk to health or the environment." This language for exempting classes of experiments is used in proposed legislation (H.R. 11192) recently reported out of the Committee on Interstate and Foreign Commerce and the Committee on Science and Technology of the U.S. House of Representatives.

In addition to these comments pertaining to the standards for exemption in the PRG-RAC, the following were directed toward the processes whereby exemption would be made:

- Rather than compile a list of non-novel exchangers exempt from the Guidelines, the burden of proof should be on the Director to compile a list of novel exchangers that are subject to the Guidelines.
- The procedures and criteria used in the development of the list should be thoroughly explained, and adequate opportunity should be given for public review and comment.
- Before an organism is placed on the list, all the data pertaining to the application should be available for public review.

In response to these comments, the PRG-NIH specifies that for exemptions I-E-4 and I-E-5—the two that involve the development of "lists"—the Director, NIH, on the advice of the RAC, will develop lists after appropriate notice and opportunity for public comment. The initial list proposed in Appendix A to the PRG-NIH is submitted for public comment along with the entire revision of the Guidelines. In the future, appropriate notice and opportunity for public comment will precede any additions to Appendix A or exemption I-E-5.

Prohibitions

Two changes in this section have been initiated to make it more compatible with the new "Definition" and "Exemptions" sections. The first was to transfer this section from Part III of the Guidelines to Part I, reemphasizing that the exemptions are not applicable to the six activities listed as prohibited. The second was to drop all references to novel recombinant DNA's and natural genetic exchange. Other alternatives suggested by commenters:

- There was a general endorsement for the provision in this section which grants to the Director, NIH, upon the recommendation of the RAC, the authority to waive any of the prohibitions. The widespread support for this authority reflects the realization that many important risk-assessment experiments would not be able to proceed otherwise. NIH is now supporting and will continue to support experiments that will yield knowledge contributing to a better understanding of the nature of potential risks of recombinant DNA.
- It was urged that the advice of other Government agencies, such as the Environmental Protection Agency and the Occupational Safety and Health Administration, should be sought when the Director, NIH, considers invoking this waiver authority. The Federal Interagency Committee on Recombinant DNA Research provides for coordination of policies in this area. EPA and OSHA are represented on the Committee. The advice of relevant research and regulatory agencies will continue to be sought when appropriate.

It was suggested that the RAC as presently constituted should not be the sole advisory body because societal as well as scientific considerations must enter into the waiver decision. As explained in greater detail in Part IV of this document, the membership of the RAC will be broadened modestly

as needed for expertise, but provision for public notice and opportunity to comment, and other appropriate administrative practices, can be used to insure adequate public input when the issues warrant.

● It was suggested that an Environmental Impact Assessment or Statement should accompany each waiver. Waiver decisions will include a careful consideration of the potential environmental impact, and certain decisions may be accompanied by a formal assessment or statement. This must be determined on a case-by-case basis.

● It was suggested that waiver of the prohibition on the large-scale use of cultures containing recombinant DNA's be issued on the basis of industry's experience in dealing with such cultures. While experience will surely be weighed in the decisionmaking, it whould not be the sole criterion for granting such a waiver.

● Agricultural scientists noted the importance to their research community of being allowed eventually to release organisms containing recombinant DNA into the environment. When the original Guidelines were proposed to the NIH Director in draft form in 1976, the release of organisms containing recombinant DNA molecules into the environment was to be allowed if a series of controlled tests had been done to leave no reasonable doubt of safety. This waiver provision was rejected at that time because of the limited scientific evidence available that any of the potential benefits from such a release were near realization. As now proposed, the prohibition of deliberate release into the environment of recombinant-DNA-containing organisms can be waived if all the requirements for a waiver (and those of the National Environmental Policy Act) are met. Given the limited experience of NIH in agricultural research, the U.S. Department of Agriculture would have to be deeply involved in this process, and written notice of this suggestion has been given to the appropriate officials at USDA.

● The Standing Advisory Committee on Recombinant DNA Research of the European Molecular Biology Organization (EMBO) has noted that the list of pathogenic organisms under prohibition I-D-1, especially those in Class 5, may not be appropriate for all European countries. The decision as to which pathogenic organisms should be classified as too dangerous to work with must be the responsibility of national or regional authorities. EMBO has recommended that a footnote be added to the Guidelines stating that the prohibition of etiologic agents relates to research in the United States. Such a footnote, however, is unnecessary because those Guidelines are directed to NIH grantees and contractors, almost all within this country.

● A final change in the PRG-NIH relates to prohibition I-D-1. As discussed in detail in the "Decision" accompanying the PRG-NIH, the prohibition against using in recombinant DNA experiments vesicular stomatitis virus and oncogenic viruses classified by the National Cancer Institute as "moderate risk" has been lifted.

Proposed Action: Environmental Impact Assessment

Part I of the PRG-NIH as discussed in this Assessment rests on the past 2 years of scientific developments relating to the safety of recombinant DNA research. The proposed alternatives to the current Guidelines reflect that evidence.

The suggested definition in the PRG-RAC spoke to the conclusion that recombinant DNA molecules only pose a potential hazard when placed in an organism, and that events in the laboratory which mimic those in nature pose no special hazard to workers or the environment. The committee also recommended, and there was strong public support for, a mechanism to allow exceptions to the "Prohibitions," as for the conduct of risk-assessment research.

The PRG-NIH also incorporate a number of the alternatives to the 1976 Guidelines suggested by the public commentators. All of the changes reflected in the PRG-NIH permit research to go forward which would not have a significant effect on the environment. Indeed, additions have been made to insure that the safety standards are extended under certain circumstances to meet environmental and occupation concerns.

The scope of the Guidelines continues to be limited to recombinant DNA research. Other techniques in genetic research, such as cell fusion and chromosomes transfer, may result in the formation of recombinant molecules; but inherent in these techniques is a range of natural barriers to the formation of hazardous organisms, and thus the issuance of Federal standards is unnecessary. It should also be noted that such techniques have been used in the laboratory for decades with no harmful effects on either health or the environment.

The definition in the PRG-NIH clearly states that the Guidelines are intended to pertain to the construction and handling of naked recombinant DNA molecules as well as organisms and viruses containing such molecules. This is to insure that the prohibitions on the use of naked DNA remain in effect. And the definition now explicitly includes synthetic molecules under the standards of the Guidelines, thereby increasing the margin of safety.

In response to the recommendations of public commentators, the PRG-NIH now require that all recombinant DNA research at NIH-funded institutions comply with the safety standards of the Guidelines and be under the purview of local institutional biohazard committees. Again, this will minimize the environmental impact, if any, of this research.

The list of exemptions, as previously explained, provides that certain recombinant DNA research need not be subject to the control of the NIH Guidelines on the basis of safety to the laboratory personnel, to the greater community, and to the environment. The proposed exemptions in large part are responsive to the great number of comments received. The "Prohibitions" section, in the 1976 Guidelines under Part III, has been transposed to Part I in the PRG-NIH to insure that none of the "Exemptions" apply to the "Prohibitions." Thus, once again, care has been taken to minimize the possibility of environmental impact.

Under the first exemption, experiments with recombinant DNA molecules that are not in an organism need not be subject to the Guidelines. This is based on the safety of these experiments. The second exemption permits certain "self-cloning" experiments to be done outside the jursidiction of the Guidelines. Again the basis is occupational and environmental safety. The third exemption also concerns "self-cloning" and permits certain experiments previously classified as P1+EK1 to be excluded from the Guidelines. This was strongly endorsed by several public commentators on the basis of no hazard.

The fourth exemption deals with experiments that mimic exchanges already occurring in nature. It allows certain of the experiments previously classified at P1+EK1 to be excluded from the Guidelines, again with the strong endorsement of several public commentators on the basis of no hazard. The final provision allows exemptions when it is found that experiments do not present significant risk to health or the environment. This standard was expressly recommended as the basis for exemption by several public commentators, especially from environmental groups, and is directly responsive to the concerns that there be an explicit reference to health or the environment for the basis of exemption. Further, in the exercise of this and the previous exemption, several procedural requirements have been introduced that will afford significant opportunity for public comment to insure appropriate attention

to occupational and environmental concerns.

A waiver provision in the section on "Prohibitions" will permit NIH support and conduct of risk-assessment experiments of crucial importance to the determination of the safety of this work. Recommendations from the scientific community, the public, and relevant Federal agencies will be sought for their advice on specific projects. Waiver decisions will include a careful consideration of potential environmental impact.

In summary, a number of safety standards and procedural requirements have been included in the PRG-NIH to insure minimal environmental impact. All experiments exempted from the Guidelines are of minimal speculative risk and present no significant hazard to health or the environment.

II. Containment

Analysis of Current Guidelines

Two approaches to the problem of containing potentially hazardous organisms form the basis of the safeguards recommended by the guidelines. Each may be viewed as setting up barriers between the laboratory worker and the organisms and between the laboratory and the greater environment. The first of these approaches involves the limitation of the actual physical escape of organisms and is referred to as "physical containment." The second approach is the use of biological barriers, to be described later as "biological containment."

(The October 1977 EIS on the current guidelines, in response to comments received on the draft EIS, documents in considerable detail the adequacy of the containment requirements and shows the bases on which judgments in this regard have been made.)

Physical containment

A major aspect of physical containment is the set of standard microbiological practices that have been developed over many years and are widely used for handling pathogenic organisms. In the hands of well-trained personnel, these procedures have proved to be effective both in laboratory and clinical settings. A second major aspect of physical containment involves the use of special kinds of equipment and facilities (1) for limiting the spread of aerosols, (2) for decontamination and containing laboratory air and wastes, and (3) for restricting access to laboratories. As with standard microbiological techniques, the type of equipment and facilities are not new but have been developed and used previously for containment of known pathogens.

The guidelines go into some detail concerning the practices and facilities required for physical containment. Four levels are specified: P1, P2, P3, and P4, in the order of increasing safeguards. P1 consists in the use of the standard microbiological practices mentioned above. P2 and the next higher level, P3, require special procedures and facilities designed to limit to increasing extents any possible accidental escape of potentially hazardous organisms. Finally, P4, the maximum level of containment, requires sophisticated and isolated facilities designed for maximum containment.

Each of the levels from P2 through P4 assumes that the standard microbiological practices demanded by P1 will also be followed. Furthermore, for each level, relevant training of personnel is mandatory. The training is to include the nature of the potential hazards, the technical manipulations, and instruction in the biology of the relevant organisms and systems. Specific emergency plans, to be used in case of accident, are required; and serological monitoring is to be provided where appropriate.

Biological containment

Biological containment is defined as the use of host cells and vectors with limited ability to survive outside of very special and fastidious laboratory conditions that are unlikely to be encountered by escaped organisms in natural environments. This is an integral part of the experimental design, since the host and vector will need to be chosen, in any given experiment, with a view to the purpose of the experiment as well as to containment.

The guidelines stress that physical and biological containment procedures are complementary, each serving to control any possible failure in the order. The use of both in a given experiment affords much higher levels of containment than either alone. Therefore, the guidelines always recommend both a particular level of physical containment and a level of biological containment for any given experiment. The guidelines explicitly recognize that better physical containment capabilities are likely to evolve as research proceeds and may reduce the needs for the standard physical containment procedures. Such innovations are to be considered as part of the on-going review of the guidelines for appropriate revision.

The Use of Bacterial Hosts and Vectors. In recognition of the relation between the host-vector system required by the experiment and the design of suitable biological containment, experments using the same host-vector system are grouped together. At present, the system of choice for many experiments is the common laboratory bacterium *E. coli*, strain K-12, and independent genetic elements (plasmids and bacteriophage) known to reside or replicate in this strain.

There are several factors contributing to this (discussed more fully in part III). Strain K-12 has been studied extensively and can be readily manipulated for recombinant DNA experiments. This extensive experience and ease of manipulation permit modification of *E. coli* K-12 and vectors used with it by classical genetic techniques for the purpose of establishing biological containment.

The guidelines discuss arguments against as well as for the use of *E. coli* K-12. The main argument against it is the intimate association of various other strains of *E. coli* with humans. By reason of the prevalence of *E. coli* strains (but not K-12) in mammals, the guidelines recommend the cautious use of *E. coli* K-12 host-vector systems and urge that efforts be made to develop alternate hosts and vectors.

E. coli K-12 appears to be harmless: It does not usually establish itself in the normal bowel or multiply significantly in the alimentary tract. These facts suggest that accidental ingestion of a small number of bacteria by a laboratory worker would not result in their extensive spread outside the laboratory. This property of the organism may be altered, however, when the infected person is taking antibiotics or has certain abnormal digestive conditions, and it is recommended that such persons not work for the duration of the abnormal circumstances.

While *E. coli* K-12 does not establish itself as a growing strain in the normal bowel, it does remain alive during its passage through the intestinal tract. Therefore, transfer of plasmid or bacteriophage vectors containing foreign DNA from the original *E. coli* K-12 host to bacteria resident in the intestines or encountered after excretion must be considered. The guidelines take into account the transferability of certain vectors in recombinant research. In brief, host-vector systems derived from *E. coli* K-12 and certain plasmids or bacteriophage appear to have extremely limited ability to spread recombinant DNA molecules.

Considering, then, the properties of *E. coli* K-12 and the known plasmid and bacteriophage vectors, the guidelines conclude that recombinant DNA's manipulated through such host-vector systems are unlikely to be spread by the ingestion or dissemination of the few hundred or thousand bacteria that might be involved in a laboratory accident, given standard microbiological practice. Therefore, these existing systems, and analogous combinations of *E. coli* K-12 with other vectors and bacteriophage, are judged to offer a moderate level of bio-

logical containment and are defined as EK1.

As with physical containment levels, increasing numbers specify increasing levels of biological containment for *E. coli* systems. For the next level, called EK2, host-vector combinations must be demonstrated to provide a high level of biological containment by suitable laboratory tests. Such combinations are obtained by genetic modification of either *E. coli* K-12 host cells or the relevant plasmids and bacteriophage or both. Various examples of the types of necessary modifications are suggested in the guidelines.

One additional level of contained *E. coli* host-vector systems, defined in the guidelines, is called EK3. EK3 systems are EK2 systems for which the specified containment properties have been demonstrated, not only by microbiological and genetic analysis but by appropriate tests in animals, including humans or primates and other relevant environments.

EK2 and EK3 host-vector systems must be certified as such by the Director, NIH, after evaluation and recommendation by the Recombinant Advisory Committee.

Alternatives: RAC-Proposed Revisions

Physical containment

Two major changes were proposed in the physical containment section of the PRG-RAC. One deals with the organization of the section; the other incorporates into the PRG-RAC the philosophy and guidance of the report of the NIH/European Molecular Biology Organization (EMBO) workshop on parameters of physical containment.

Physical containment requirements for each P-level have been organized under the topic headings "Laboratory Practices," "Containment Equipment," and "Special Laboratory Design." This was done to emphasize the importance of laboratory practices and containment equipment in achieving the desired safety objective.

Other proposed revisions contained in the physical containment section reflect a conscious effort to encourage international uniformity with respect to recombinant DNA guidelines. This has been achieved by revising the containment descriptions so that they are consistent with the guidance provided in the NIH/EMBO report. In addition, some statements have been rewritten and others added in order to clarify the basic requirements for each level of containment. The most significant clarifications have been made in the areas on containment equipment and special facility design. The revisions, however, have not resulted in changing the purpose or intent of the physical containment descriptions in the 1976 guidelines.

One specific addition to the PRG-RAC that has originated from the NIH/EMBO report is the inclusion of design criteria for an area of the laboratory in which personnel wear positive-pressure suits ventilated by life-support systems. This added approach provides a level of physical containment equivalent to that afforded by glove-box cabinets at the P4 level.

Other important recommended changes include—

- Certain good microbiological practices are mandatory at the P1 level in the PRG-RAC (the 1976 guidelines encourage but do not require such practices);
- At the P2 level, prohibitions against eating, drinking, smoking, and storage of foods have been extended from the work area to the entire laboratory;
- The universal biohazard sign is now required at the P2 level, and its use has been extended to equipment such as freezers and refrigerators in which organisms containing recombinant DNA molecules are stored;
- Access procedures have been specified for controlled areas adjacent to P3 laboratories;
- Installation of foot-, elbow-, or automatically-operated facilities for washing hands is now required for all laboratories in which P3-level work is done;
- Specific guidance on containment equipment appropriate for laboratory animals has been added to the P3 and P4 sections; and
- The labeling requirements for shipment of etiologic agents now apply to all organisms containing recombinant DNA molecules. Thus, the Center for Disease Control, U.S. Public Health Service, must be notified in the event of any accidental breakage during shipment. Also, agents requiring P4 containment must be packaged according to strict Federal standards and shipped by registered mail or an equivalent system that provides for sending notifications to the shipper upon delivery.

The PRG-NIH adopt these suggestions in large part. Thus, they strengthen the safety standards and procedures for physical containment and move toward international agreement.

Biological containment

The PRG-RAC describe the categories of hosts and vectors to be used in minimizing the spread of organisms containing recombinant DNA. The PRG-RAC differ from the 1976 guidelines in that they were expanded to include (1) further definitions of host-vector systems, (2) a more restrictive set of requirements for HV3 systems (see below), and (3) a new section describing mechanisms for certification.

Definitions of Host-Vector Systems. A new nomenclature—HV1, HV2, and HV3—was developed to incorporate a variety of hosts and vectors into the framework initially established for *E. coli* K-12. In particular, the PRG-RAC provide criteria for HV1 systems other than *E. coli* K-12. In the 1976 Guidelines, cloning systems other *E. coli* K-12 were to be considered only if superior to K-12 in containment properties; but it is now recognized that many useful experiments can only be conducted using host-vector systems other than those based on *E. coli* K-12, and that such experiments should be permitted so long as the proposed system provides equivalent biological containment. The new HV1 criteria provide a structure for approval of systems that meet these requirements.[2]

Requirements for HV3 Systems. These have been made more stringent than the corresponding requirements for EK3 in the 1976 guidelines. The PRG-RAC require that the vector be dependent on its propagation host or be highly defective in mobilizability. Also, it may carry no markers conferring resistance to antibiotics used clinically or in agriculture. This provision should preclude any inadvertent advantage for recombinant-DNA-bearing vectors that encounter antibiotics in the environment.

Certification of Host-Vector Systems. A new section has been added detailing the responsibility for certification of HV1, HV2, and HV3 systems, the types of data to be submitted, and the mechanisms for distributing strains once certified. The section delineates procedures used by the RAC for the past 2 years and therefore represents no change from practices under the 1976 guidelines.

These recommendations, reflected in the proposed revisions, set improved procedures and standards to meet environmental and occupational concerns.

Physical and biological interaction

Another specific addition to the guidelines that has originated from the NIH/EMBO report is particularly notable—the addition of tables I and II to the P3 and P4 sections, respectively. These tables show combinations of physical and biological safeguards that provide similar protection. This approach allows flexibility in selecting comtainment equipment for a particular study without compromising safety.

Alternatives: Public Commentators

Commentators have expressed particular concern over (1) the flexibility

[2] Under the proposed revisions, new HV1's need not offer a distinct advantage over *E. coli* K-12 host-vectors, need not be capable of modification to HV2 and HV3, and need not be class 1 etiologic agents.

that allows various combinations of containment safeguards, (2) the adequacy of risk-assessment studies in relation to physical containment, (3) the adequacy of training in laboratory safety practices, (4) plans for dealing with emergencies, and (5) various aspects of the biological safety concepts. NIH has considered a number of recommendations by EMBO. Public commentators have made additional suggestions relating to actions at specific levels of physical containment and to shipment of recombinant DNA materials.

Concept of "flexibility"

Some commentators have expressed concern over the flexibility provided in tables I and II which allows various combinations of containment safeguards. Some feel, for example, that work in a P3 facility conveys a desirable sense of hazard, whereas a reduction to the P2 level will promote an undesirable relaxation of vigilance. It has also been suggested that an increase in the options augments the difficulty of control and implementation. Some commentators object to specific options provided at the P3 and P4 levels.

NIH has been urged to include a better explanation of the rationale for flexibility. Indeed, the calculus of switching physical and biological containment levels has been questioned. Is reducing a physical containment level from P3 to P2 truly compensated by increasing biological containment from EK1 to EK2?

The scale of either form of containment from least to greatest is not necessarily linear, and substitutions are only roughly approximate. Nevertheless, there are some numerical bases for comparison. At the P4 level, for example, a Class III biological safety cabinet is required (if a positive-pressure suit is not used); whereas one can work in an open-front biological safety cabinet at the P3 level. Studies using molecular counts of gases have shown that there is a 4-5 log protection in going from a Class I or II biological safety cabinet to a Class III cabinet—that is, from P3 to P4. Similarly, in going from P1 to P3 there may be a 4-5 log increase in safety.

The measure of safety provided by open-front biological safety cabinets, used in work at the P2 and P3 levels, consists in design and performance criteria that permit fewer than 20 microorganisms to escape through the open front when 1×10^8 to 8×10^8 (100,000,000 to 800,000,000) microorganisms are experimentally released within the cabinet. (See pp. 92-93 of the 1977 EIS for a more detailed discussion of safeguards associated with use of biological safety cabinets.)

HV2 is defined in terms of a probability of escape of recombinant DNA of less than 10^{-8} (1 in 100,000,000). In considering "equivalency" between P and EK levels, it is recognized that the two systems are conceptually different. Biological safety cabinets are designed primarily for the protection of the laboratory worker, and all physical containment protection stops at the walls of the laboratory. Biological containment continues to operate even if an organism should escape from the laboratory.

The flexibility allowed in alternate P and HV levels are carefully explained in the text of the PRG-NIH, and the investigator must follow the explicit requirements set forth in Part III of the PRG-NIH and in Tables I and II.

Risk assessment

Many commentators have urged more studies in risk assessment. It has been maintained that assumptions about biological containment may not be valid and that all components should be tested. Concern has been expressed that the biological containment safety systems may fail altogether.

Some risk-assessment studies are prohibited by the 1976 Guidelines. Under the PRG-RAC, however, the Director, NIH, on recommendation of the RAC, would have discretion to permit such risk-assessment experiments by granting a waiver from a specific prohibition. There was virtually unamimous support for this discretion at the public hearing in December 1977. Of course, its exercise must be consistent with standards of due process for the scientific community and the public.

Risk-assessment studies are proceeding both within and outside the United States. NIH is committed to the conduct and support of such studies to determine the extent to which certain potentially harmful effects from recombinant DNA molecules may occur. It is intended that the NIH P4 facilities at the Frederick Cancer Research Center will serve as a focal point for such experiments. Provisions have already been made to share these facilities with non-Federal scientists.

Training

A number of commentators urge that specific curricula be developed for training of researchers and that the Guidelines stipulate requirements for certification in safety practices. NIH has a contract with the American Society for Microbiology to develop minimum standards for training participants in recombinant DNA research. The work panel's report is to be used by the IBCs and investigators to set appropriate standards.

Emergency plans

In response to the concerns of commentators, the elements of emergency plans to handle possible safety problems are described more clearly in "Laboratory Safety Monograph—A Supplement to the NIH Guidelines for Recombinant DNA Research." Further, NIH staff have recently met with representatives of CDC to establish a mechanism for providing advice, consultation, or assistance if necessary in case of an emergency, such as a laboratory accident involving recombinant DNA.

Biological containment considerations

Considerations of biological containment related mainly to the development of alternative host-vector systems. Many commentators from the scientific community believe that the PRG-RAC discriminate against host-vector systems alternate to *E. coli* K-12. They urge development of other systems, maintaining that they will be needed increasingly both in pure research and in industry and should be certified as soon as possible. It is unlikely, according to one commentator, that agriculture will best be served through the use of *E. coli* K-12 (or *B. subtilis*) and that alternate host-vector systems are essential if the potential of recombinant DNA technology for agriculture is to be realized. In view of the support evident at the 1976 public meeting for NIH to encourage development of alternate host-vector systems, one commentator expressed disappointment that there was not a large NIH contract program in this area.

Others view the introduction of alternate HV systems with some misgivings. It was pointed out, for example, that if uncertainty continues to surround research with so well-studied an organism as *E. coli* K-12, our ignorance must be that much greater with regard to any other organism—its ecological involvement, the organisms with which it can exchange DNA, etc. Moreover, the Guidelines, which have been developed around the use of *E. coli* K-12, are primarily focused on dangers to man, and the introduction of new systems may affect other life forms with which we should be equally concerned. In the view of commentators who urge restraint, the larger the number of systems certified, the greater the problem of monitoring the work.

Clearly, however, research address to the development of other host-vector systems must proceed. This is particularly evident in the agricultural sector, where the potential for immediate benefits to man is great. At present, a number of alternate systems are being developed by NIH grantees. In the use of such systems, the same con-

siderations of safety and risk associated with the use of *E. coli* K-12 will, of course, apply.

Mouth pipetting at the P1 level

Both the 1976 guidelines and the PRG-RAC prohibit mouth pipetting at the P2, P3, and P4 levels. For the P1 level, however, they state, "Although pipetting by mouth is permitted, it is preferable that mechanical pipetting devices be used." A number of commentators have urged that mouth pipetting be prohibited at the P1 level of physical containment. The decision accompanying the PRG-NIH discusses in detail the arguments of various groups on both sides of the issue. In resolving this, the Director, NIH, has decided to adopt the conservative position and ban mouth pipetting. Accordingly, language has been inserted in the PRG-NIH saying that at the P1 level "mechanical pipetting devices shall be used; pipetting by mouth is prohibited." Since mouth pipetting had already been banned at the P2-P4 levels, this means that mouth pipetting would now be banned for all experiments covered by these guidelines.

Proposed Action: Environmental Impact Assessment

Physical containment

The PRG-NIH include the following specific changes at each P level, aimed at clarifying and strengthening physical containment requirements and thereby reducing the probability of recombinant DNA molecules being accidentally released into the environment.

P1 Level. The 1976 guidelines establish as a principle of containment the adherence to good microbiological practices. At the P1 level, however, certain practices are not required but merely encouraged. The PRG-NIH now make adherence to certain of these practices mandatory at all four physical containment levels.

A major change in the PRG-NIH is the banning of mouth pipetting at the P1 level, meaning that mouth pipetting is now banned for all experiments covered by the guidelines. Since the only plausible way *E. coli* K-12 could gain entry into laboratory workers is by oral ingestion, this ban greatly reduces the possibility that any organisms containing recombinant DNA will escape and thus minimizes the risk of environmental impact.

P2 Level. The PRG-NIH have extended the prohibitions against eating, drinking, smoking, and storage of foods from just the work area to the entire P2 laboratory. This change was made to achieve consistency with recommended practices in laboratories where research with low-risk human pathogens is conducted.

The PRG-NIH call for posting of the universal biohazard sign on storage freezers and refrigerators. This additional requirement was specified to facilitate safe storage of organisms containing recombinant DNA molecules when research with them is not in progress.

P3 Level. In the PRG-NIH, a controlled access area is defined as one that separates the P3 laboratory from areas open to unrestricted traffic flow. Access procedures for this area are specified, clarifying the juxtaposition of the P3 laboratory and uncontrolled areas.

The PRG-NIH require the installation of foot, elbow, or automatically operated facilities for washing hands in laboratories in which P3-level work is conducted. This additional requirement was made to eliminate the possibility of contaminating faucets through contact. The requirement is consistent with practices common to infectious disease laboratories.

The PRG-NIH would permit recirculation of untreated air within individual rooms of the P3 laboratory. Such recirculation can conserve energy without compromising safety. Reference to recirculation of treated air to other areas of the building has been eliminated, because this approach to energy conservation is generally not practical.

Some commentators pointed out that the PRG-RAC did not require an autoclave in the P3 laboratory itself but only within the building. It has been suggested that the autoclave should be as close as possible to the controlled area of the P3 laboratory. The language of the 1976 guidelines, stating a preference for the autoclave to be within the controlled laboratory area, is therefore retained in the PRG-NIH. A requirement, however, that the autoclave be within the controlled area would increase costs, and in the view of NIH would not add measurably to safety.

P4 Level. In the PRG-NIH the minimal age for entry into a P4 laboratory has been raised to 18 years, the commonly accepted legal age.

The PRG-NIH specify containment equipment appropriate for the isolation of experimental animals. This was added because the lack of guidance in the current guidelines has led to confusion in their application to animal experimentation.

The PGR-NIH provide flexibility in selecting combinations of physical and biological containment to be used for a given experiment. All possible combinations available for selection achieve P4-level safety objectives. This approach was patterned after the guidance provided in the NIH/EMBO report.

The PRG-NIH specify design criteria for a suit area where personnel wear positive-pressure suits that are ventilated by life-support systems. This provides an option to the class III cabinet-system requirement at the P4 level of physical containment. This option has been used successfuly in research with extremely hazardous human pathogens. Without compromising safety, it provides an opportunity to conduct research procedures that cannot be confined to conventional class III cabinet systems.

Shipment. In the PRG-NIH, the labeling requirements for the shipment of etiologic agents apply to all organisms containing recombinant DNA molecules, rather than to just those containing molecules derived from an etiologic agent listed in 42 CFR 77.25. This change was made in order to insure that the Center for Disease Control, U.S. Public Health Service, would be notified in the event of accidental breakage during shipment. The PRG-NIH also specify that agents requiring P4 physical containment must be shipped by registered mail or an equivalent system that provides for sending notifications to the shipper immediately upon delivery. This change would impose on the shipment of organisms requiring P4 containment the same standards used for shipment of high-risk human pathogens.

Biological containment

A number of alternate systems for biological containment are being developed by NIH grantees, including ones using *Bacillus subtilis* and *Saccharomyces cerevisiae.* To assure adequate safety control, a new section of certification of host-vector systems has been added to the guidelines. HV1 systems other than *E. coli* K-12 are reviewed by an expert working group, then by the RAC, which makes appropriate recommendatons to the Director, NIH. Descriptions of the organism, its biology, and the characteristics of the particular strains to be used are considered. The same standards of safety and risk associated with the use of *E. coli* K-12 will apply to any new host-vector systems to be certified in the future.

HV2 Level. For the HV2 level of containment, the RAC, on June 23, 1977, unanimously approved a document entitled "Instructions to Investigators Concerning Data To Be Submitted on Host-Plasmid Systems Proposed for EK2 Certification." Although not officially part of the PRG-RAC, this document sets forth criteria that any putative EK2 host-vector systems must meet before recommendation by the RAC for certification (see appendix H of the October 1977 EIS). The committee applied these criteria in reviewing new systems (pBR322 and pBR313 in

x1776) at the June 23 meeting, and will do so for all future submissions. These criteria are clearly more stringent than previous ones, and this means that EK2 host-vector systems approved now and in the future will be even safer than those approved previously.

HV3 Level. Requirements for HV3 systems have been made more stringent that those in the 1976 guidelines. The additional requirements mean that only some HV2 systems are eligible for consideration as HV3 systems. This should significantly increase the containment of HV3 systems and therefore increase the safety of experimentation. In addition, attempts to develop HV3 systems that meet these criteria should simultaneously upgrade the HV2 systems in use, since it is to the experimenter's advantage to use the HV2 systems that have the greatest likelihood of meeting HV3 criteria.

Certification of HV Systems. A new section has been added to clarify responsibility for certification of all host-vector systems, the types of data to be submitted, and the mechanisms for distributing strains once certified. The section delineates procedures used by the RAC for the past year and thus represents no change from practices under the 1976 guidelines.

Under the PRG-NIH, HV1 systems other than *E. coli* K-12 and HV2 and HV3 systems are considered by an expert working group and then by the RAC, which makes appropriate recommendations to the Director, NIH. Modifications of HV2 and HV3 systems must be independently certified by NIH. Data to be submitted are detailed. All HV2 and HV3 systems are to be obtained from NIH or its designee, and recipients are to report to NIH any discrepancies from the expected properties. If the strains are propagated by NIH, a sample will be tested for relevant properties prior to distribution. The requirements assure adequate controls in the certification and distribution of host-vector systems and provide sufficient protection against potential hazards to the environment.

Containment Properties of Hosts and Vectors. In regard to containment properties of individual organisms used in recombinant DNA research, recent experimental evidence supports the view that biological containment works well. This is particularly borne out by results from experiments specifically designed to test the survivability and colonizing ability of *E. coli* K-12 and EK2 host-vector systems and the transmissibility of plasmids and phage vectors.

At the time of the release of the current guidelines in 1976, EK2 systems were defined but none existed. An extramural contract program was initiated to develop safer host-vector systems and to verify their genotypic constitution and phenotypic traits. The program is administered by the National Institute of Allergy and Infectious Diseases. The ability of these systems to survive in laboratory and natural environments was determined. As a result of this contract program and of work by other investigators, a series of EK2 host-vector systems was developed. The RAC subjected these to great scrutiny and finally recommended them for certification. A list of certified EK2 host-vector systems appears in appendix H of the October 1977 EIS.

III. Containment Guidelines for Covered Experiments

Analysis of Current Guidelines

E. Coli *K-12 Host-Vector Systems*

The several levels of physical and biological containment are defined in part II, and specific recommendations are given for experiments using the *E. coli* K-12 host-vector systems. Each type of experiment is assigned both a physical containment level (that is, a P level) and a biological containment level (an EK level). The particular combination of the two reflects the severity of the estimated potential hazard.[3]

The guidelines are organized, for the *E. Coli* systems, according to the source and nature of the foreign DNA. A sample of DNA containing essentially all the genetic information of an organism can be isolated and fragmented. If the experiment involves such a mixture of DNA fragments, it is referred to as a "shotgun" and will call for a certain level of containment. Experiments involving such mixtures of DNA fragments are assumed to be of higher potential hazard than those done with a single, purified fragment, because of the greater likelihood of dangerous and unknown genes being introduced into a recipient cell. Purified fragments containing mainly genes whose properties are known and are not harmful offer less potential hazard than a shotgun experiment.

In some instances, the foreign DNA will be derived from extra chromosomal genetic elements. Such elements include the DNA of animal viruses, plant viruses, other eukaryote organelles such as mitochondria and chloroplasts, as well as prokaryote plasmids or bacteriophages of the same type used as vectors. Each of these cases is treated separately in the guidelines. The prokaryote sources are treated differently, depending on whether the "foreign" DNA is from an organism that does or does not exchange genetic information with *E. Coli* in nature.

The physical and biological containment is listed for various possible DNA sources: both must be used, as they complement each other. For example, DNA from primates requires the most stringent containment, since the estimated potential hazard, either from genes that might function in humans with untoward effects or from pathogenic viral DNA's residing in primate tissue, is judged to be most serious. The experiments now require either P3 + EK3 or P4 + EK2; and it should be noted that only the latter combination is feasible at present, and then only at the limited number of P4 facilities.

In two instances—primates and cold-blooded vertebrates—containment requirements are lower if the DNA is isolated from embryonic tissue, or germline material, since such material is less likely to be contaminated by pathogenic viruses than is adult tissue. Thus, if the foreign DNA is from cold-blooded vertebrates, P2 and EK2 are required, but P2 and EK1 can be used if the DNA is from embroyonic or germ-line tissues. If the cold-blooded vertebrate is known to produce a potent toxin, P3 and EK2 must be used. In some instances—lower eukaryotes, for example—the guidelines require more or less stringent conditions, depending on whether or not the source of foreign DNA is known to be pathogenic or toxigenic, or might be infected with a pathogen, or is known to make a harmful product.

The guidelines for shotgun experiments, when the source of the DNA is a prokaryotic organism, may be summarized. First those prokaryotes that are known to exchange genetic information with *E. Coli* in nature are considered. The containment requirements are low for this group, since it is unlikely that the experiments will create new genetic combinations. Requirements vary with the pathogenicity of the source of foreign DNA. When the source is a prokaryote that does not naturally exchange genetic material with *E. Coli*, the containment recommendations are high, for there is a greater potential for new genetic combinations to be formed and expressed. Further, it is assumed that the more similar the DNA's of donor and host, the greater the probability of expression of foreign DNA or of derepression of host genes.

Characterized clones obtained from shotgun experiments may not be as potentially hazardous as the original mixture of cells. Cloning of the recipient host cell containing the DNA fragment of interest will be one of the normal aims of any recombinant DNA experiment. The guidelines state that

[3] In the PRG-NIH the EK systems are included in the broader HV (host-vector) categories created to accommodate potential systems ranging beyond *E. Coli* K-12.

experiments involving a clone derived from a shotgun experiment can be done under P1 + EK1 conditions if the clone has been rigorously characterized and is free of harmful genes and if the foreign DNA was from a species that exchanges genes with *E. Coli* in nature. If the foreign DNA was from a species that does not do so, then P2 + EK1 is required.

Similarly, when the initial recombination involves a purified segment of the foreign chromosomal DNA, rather than a mixture, the potential for growth of a hazardous organism will be less, since the number of clones that must be examined to obtain the desired clone is markedly reduced. If certain criteria for purity are met, the investigator may lower the containment conditions from those recommended for shotgun experiments with DNA of the same source by one step either in physical containment or biological containment. Thus, for example, shotgun experiments with DNA from birds require P3 + EK2. A DNA fragment from birds that is free from harmful genes, and 99-percent purer before being joined to a vector, would require either P2 + EK2 or P3 + EK1.

The final group of *E. Coli* experiments considered are those in which the foreign DNA is itself from an extrachromosomal element. It is assumed that such DNA is purified away from chromosomal DNA before recombination. For example, DNA from all or part of the genome of an animal virus requires P4 physical containment and an EK2 host-vector system, or P3 + EK3. If the recombinants have been purified by cloning and shown to be free of harmful regions of the viral genome, then experiments can be at P3 + EK2.

When complementary DNA's, synthesized *in vitro* from RNA preparations, are used in recombinant DNA experiments, the containment requirements are as described for isolated DNA preparations.

Animal host-vector systems

Many recombinant DNA experiments will involve the use of systems in which the host cells are eukaryotes grown as single cells in tissue culture. Useful vectors may include extrachromosomal DNA elements, such as the DNA of organelles or viruses. The cells themselves are fragile and fastidious, and there is little or not chance that a living cell could escape from a laboratory in the way than an *E. coli* cell might. Therefore, containment considerations focus on the vectors.

Animal viruses can escape the laboratory in a viable form, especially if laboratory workers become infected. There are now two animal viruses whose DNA's are useful as vectors: polyoma and simian virus 40 (SV40). Both viruses are oncogenic—that is, they cause tumors in small newborn laboratory mammals. Polyoma virus, however, does not infect human cells grown in the laboratory or, judged by the lack of antibody formation, infect whole human beings. SV40 does colonize humans. Indeed, the virus contaminated the early Salk polio vaccine, and millions of people were inadvertently inoculated with in in the 1950's. To date, there is no indication that the recipients suffered any related disease. But under the Guidelines, more stringent physical containment is required for SV40 than for polyoma.

The Guidelines require that the viral DNA used for recombination with a foreign DNA must itself be defective—that is, its propagation as a virus must be dependent on the presence of helper virus that supplies the genes for the missing functions. This helper can be nondefective under certain conditions. In some experiments, no production of viral particles is required and no helper may be needed. Biological containment is inherently greater in the absence of virus particles, since cells themselves are relatively easy to contain. In experiments using a virus as vector, the particular levels of physical containment depend on the source of the foreign DNA, on whether polyoma or SV40 is the chosen vector, and whether virus particles are produced.

Plant host-vector systems

The Guidelines also cover experiments in which plant cells will serve as hosts for recombinant DNA. The cells might be single plant cells grown under laboratory conditions, or seedlings, plant parts, or small whole plants. This is the only instance where the Guidelines address the question of recombinant DNA experiments with whole organisms. Directions are given for modification of the specifications for P1, P2, and P3 containment in order to provide conditions appropriate for work with plants.

Vectors for use in experiments with plants include plant organelle DNA such as that of chloroplasts, and DNA of viruses of low pathogenicity and restricted host range. These vectors offer moderate levels of biological containment. The requirements are organized according to the source of the foreign DNA and to whether it is a species in which the vector DNA is known to replicate. P2 conditions are required if the source is not dangerous and is one in which replication of the vector occurs. If the foreign DNA is derived from a species in which the vector is not known to replicate, then requirements range up to P4 depending on whether the DNA is purified and whether it contains harmful genes.

Other host-vector systems

Theoretically, there are a variety of organisms, both prokaryotes and lower eukaryotes such as fungi and yeast, which will be useful hosts for experiments with recombinant DNA's. Some may offer the special advantage of not infecting humans, animals, or other important ecological niches. The growth characteristics of such hosts indicate that containment problems will be like those for *E. coli* K-12. The Guidelines urge development of these systems and point out that the detailed recommendations made for *E. coli* K-12 systems can serve as a guide in determining physical and biological containment requirements when necessary.

Alternatives: RAC-Proposed Revisions

A major concern for all individuals who have participated in establishing guidelines for recombinant DNA research is that any guidelines that are drafted and adopted be reassessed periodically and changed when warranted by new information. In keeping with this responsibility, the RAC has compiled additional information pertaining to risk assessment in recombinant DNA-research. The information is from the following sources:

- Consultations with scientists expert in the areas of evolution, plant biology, bacteriology, virology, and human and animal infectious diseases;
- Reports from scientific meetings dealing with the potential biohazards of recombinant DNA research (for example, Tenth Miles International Symposium on Recombinant Molecules—Impact on Science and Society, Cambridge, Mass., June 1976; National Academy of Sciences Forum on Recombinant DNA Research, Washington, D.C., March 1977; Genetic Engineering for Nitrogen fixation, Brookhaven, N.Y., March 1977; and the Workshop on Studies for Assessment of Potential Risks Associated with Recombinant DNA Experimentation, Falmouth, Mass., June 1977);
- Results from experiments specifically designed to test (1) the survivability and colonizing ability of *E. coli* K-12 and EK2 host-vector systems, (2) the transmissibility of plasmids and phage vectors, (3) the potential of *E. coli* K-12 for pathogenicity, and (4) the potential for genetic exchange between diverse bacteria and between prokaryotic and eukaryotic organisms.

Each category of experiments in Part III of the 1976 Guidelines was then extensively examined, and the following criteria were applied to the new information:

- The degree to which the DNA segment has been purified away from other genes and shown to be free of harmful characteristics,

- The potential biohazard associated with the DNA of the cell or microorganism that serves as the DNA source (e.g., genes for toxin production),
- The potential biohazard associated with the vector that serves to transmit the source DNA to a recipient host cell,
- The ability of the vector to survive in natural environments or habitats,
- The kinds and number of organisms that are susceptible to infection by the vector or recipient,
- The potential biohazard of the recipient host cell that serves to replicate the recombinant DNA molecules,
- The ability of the host cell to survive in natural environments or habitats,
- The ability of the host cell to transmit the recombinant DNA molecule to other cells capable of surviving in natural environments or habitats,
- The potential of the host cell to obtain the source DNA by natural means, and
- The evolutionary relatedness of the DNA source to humans. The potential dangers are considered to increase as the organism providing the source DNA approaches humans phylogenetically. For example, source DNA from primate cells is considered to be more potentially dangerous than that from prokaryotes.

In an effort to present more clearly the changes in containment levels proposed by the PRG-RAC, a table was prepared for use at the December 1977 DAC meeting which compared the containment levels in the original (current) Guidelines and the PRG-RAC. This table has now been expanded with a third column to show the containment levels of the PRG-NIH (Appendix A). The remainder of this section summarizes a number of the proposed changes, comparing the current Guidelines with the PRG-RAC. (Not all the changes are discussed here; certain items in which the PRG-NIH differs significantly from the PRG-RAC are discussed below under "Alternatives: Public Commentators." The numbers in parentheses indicate the line numbers on the table to which the proposed revision applies.

- Several categories of experiments (primarily those involving prokaryotes that are exchangers of genetic information with *E. coli* in nature) would no longer be subject to the Guidelines because of the changes in the definition. (See lines 20, 21, 27, 46, and 47.)
- Shotgun experiments involving birds and mammals other than primates were the subject of lowering of containment from P3+EK2 to P2+EK2. This action reflects the increased confidence of the RAC in the EK2 host-vector systems. (See lines 4 and 5.)
- Another category which the RAC decided was in need of revision was that pertaining to the cloning of DNA from organisms producing a toxic product. This was clarified in the PRG-RAC by setting containment levels according to whether polypeptide toxins are produced. Polypeptide toxins are specified, since they might be encoded by a single gene or cluster of genes, whereas toxins of other chemical structure would not. (See lines 8, 9, 10, 11, 12, 16, 17, and 19.)
- For several categories of experiments, it is proposed that the investigator have the option of working at P2+EK1 or P1+EK2 rather than the P2+EK1 levels previously specified. This again reflects confidence in the EK2 systems. (See lines 7, 14, and 15.)
- The lowering of containment for experiments with rigorously characterized clones free of harmful genes has been revised to provide more flexibility. Under the PRG-RAC, Institutional Biosafety Committees (IBC's) would be able to lower containment by a single level. The IBC should consider the purity, extent of characterization, and harmlessness of the clone before allowing such lowering. Reduction of containment by more than one level would require approval by NIH. Under the 1976 Guidelines, NIH had the option of lowering containment down to certain specified levels or not lowering it at all. The PRG-RAC would allow NIH to consider all available data for the clone and to lower containment accordingly.

Alternatives: Public Commentators

During the development of the original Guidelines in early 1976, Part III was the section most commented upon. There was also much comment on this section in the PRG-RAC. Many of the issues raised, however, did not address the specific proposals to alter the containment levels but more general topics, such as the need for a rationale for each of the changes.

Rationale

A number of commentators asked that the rationale for the classification of permissible experiments be elucidated. Concern was expressed that:

- It was difficult for a layman to understand the entire section on permissible experiments because the rationale is not detailed in either the current Guidelines or the PRG-RAC;
- The whole categorization is dependent upon investigatorial confidence rather than documented fact; and
- The quantification of containment levels, the means whereby they were decided, and the rationale for raising and lowering them is not clear.

In general, the classification is somewhat arbitrary. It depends in large part on the scientific judgment of the RAC rather than on demonstrable risk, because there is in fact no actual scientific evidence that there is a hazard in any recombinant DNA experiment. The rationale for classifying recombinant DNA experiments at several containment levels was explained in the "Decision of the Director, National Institutes of Health, To Release Guidelines for Research on Re- combinant DNA Molecules," which was published along with the current Guidelines in the FEDERAL REGISTER on July 7, 1976 (page 27908), as follows:

> The guidelines assign different levels of containment for experiments in which DNA from different sources is to be introduced into an *E. coli* K-12 host-vector system. The variation is based on both facts and assumptions. There are some prokaryotes (bacteria) that constantly exchange DNA with *E. coli*. Here it is assumed that experimental conditions beyond those obtained in careful, routined microbiology laboratories are superfluous, because any exchange experiments have undoubtedly been performed already in nature.
>
> In every instance of artificial re- combination, consideration must be given to the possibility that foreign DNA may be translated into protein (expressed), and also to the possibility that normally repressed genes of the host may be expressed and thus change, undesirably, the characteristics of the cell. It is assumed that the more similar the DNAs of donor and host, the greater the probability of expression of foreign DNA, or of possible derepression of host genes. In those cases where the donor exchanges DNA with *E. coli* in nature, it is unlikely that recombination experiments will create new genetic combinations. When prokaryote donors not known to exchange DNA with *E. coli* in nature are used, however, there is a greater potential for new genetic combinations to be formed and be expressed. Therefore it is required that experiments involving prokaryotic DNA from a donor that is not known to exchange DNA with *E. coli* in nature be carried out at a higher level of containment. Recombination using prokaryotic DNA from an organism known to be highly pathogenic is prohibited.
>
> There are only limited data available concerning the expression of DNA from higher forms of life (eukaryotes) in *E. coli* (or any other prokaryote). Therefore, the containment prescriptions for experiments inserting eukaryotic DNA into prokaryotes are based on risks having quite uncertain probabilities.
>
> On the assumption that a prokaryotic host might translate eukaryotic DNA, it is further presumed that the product of that foreign gene would be most harmful to man if it were an enzyme, hormone, or other protein that was similar (homologous) to proteins already produced by or active in man. An example is a bacterium that could produce insulin. Such a "rogue" bacterium could be of benefit if contained, a nuisance or possibly dangerous if capable of surviving in nature. This is one reason that the higher the phylogenetic order of the eukar-

yote, the higher the recommended containment, at least until the efficiency of expression of DNA from higher eukaryotes in prokaryotes can be determined.

The structure of the classification for permissible experiments is based, therefore, on the scientific assumptions governing potential risk. It should be emphasized that although a wide variety of recombinant DNA experiments have now been performed for over 5 years, in hundreds of laboratories throughout the world, no case of hazard has been demonstrated.

Part III of the Guidelines assigns to each specified class of experiments a level of physical containment and a level of biological containment at which the experiments shall be performed. As noted before, there is a 4-5 log protection in going from P1 to P3 or from P3 to P4. For biological containment, there is the criterion for the HV2 system that the chances of the recombinant DNA escaping, either via survival of the organisms or via transmission of recombinant DNA to other organisms, should be less than 10^{-8} (1 in 100,000,000) under specified conditions.

Commentators said that the revisions did not bring the Guidelines any closer to establishing absolute levels of hazard. It was brought out, however, that in the use of *E. coli* K-12, a level of no risk is neared. Data presented at the Falmouth Conference indicate that it is essentially impossible for *E. coli* K-12 to be transformed into a wild-type pathogen. An *E. coli* K-12 containing toxic genes through recombination could present a risk, for example, to the laboratory worker who ingested it. But it would only be a risk to that person.

Harmful genes will have a very low probability of being transfered from *E. coli* to another organism. For example, the plasmids at the HV2 level are engineered so that they neither self-transfer, nor transfer when another plasmid induces conjugation. Current work is designed to determine the probability of *E. coli* K-12 as a host taking up plasmids from the environment that can then receive the recombinant DNA molecules from the engineered plasmid. Recent data indicate that the probability is extremely low. Thus, it is clear that this host-vector system offers a high degree of safety and at present is preferable to any other.

Comments on use of E. coli *K-12*

A number of comments were made concerning the use of *E. coli* host-vector systems. One commentator stressed that because *E. coli* K-12 is currently a "poor" pathogen does not mean that one or two genes might not convert it to a "good" pathogen. The enfeebled nature of *E. coli* K-12 "is presumably the consequence of mutation(s) introduced during its laboratory passage," but different strains of K-12 with different histories may not all be similarly enfeebled.

Further, it was claimed that the failure to convert K-12 to a pathogen by the use of certain plasmids or *Salmonella* genes is not definitive. To be definitive, we must have the detailed nature of the mutations in K-12 that "prevent the expression of pathogenicity." Also, it was noted that there is no way to assess the absolute risk associated with these experiments, and that it is important to assess the potential harm not only to man but to plants, animals, and the environment.

Another commentator urged that this section of the Guidelines be supplemented with evidence from the Falmouth conference to show that the potential risk is minimal. A commentator cited the potential risk on the basis that "virtually any highly conserved physiologically active eukaryotic protein * * * or fragment thereof could be highly toxic when introduced out of context by a bacterium which received the appropriate gene in a recombination experiment." This criticism of *E. coli* K-12 does not detract from the scientific knowledge over the past two years of the great safety of this system. The evidence is presented in detail in the following section, "Environmental Impact of the Proposed Action."

Different strains of K-12 with different histories may not all be similarly enfeebled, and failure so far to convert K-12 to a pathogen does not prove it can never happen. However, the safety of *E. coli* K-12 has been clearly shown, and there is no need to limit or specify particular strains for EK1. After 30 years of work with many different strains, there is still no known pathogenic *E. coli* K-12 strain. Thus, there is presumptive evidence that all K-12 strains are safe. They are well suited for laboratory experiments because they take up DNA easily, but their cell wall makes them unsuited to compete in nature with wild-type *E. coli*.

Still, it is impossible to refute the criticism that absolute conclusions as to risk have not been reached. There is always one more experiment to be performed that would help in analyzing the safety aspects of any potentially hazardous research activity. Two years ago the Director, NIH, in releasing the Guidelines, stated that NIH would proceed with recombinant DNA work in a deliberately cautious manner while simultaneously evaluating all the evidence pertaining to the potential risks. That statement is reaffirmed.

General classification

There was disagreement expressed over whether the PRG-RAC were too stringent or too lax. Those arguing the former position maintain that the RAC did not relax the Guidelines enough, because all the experimental evidence gathered and analyzed in the past 2 years indicates that the initial fears concerning the potential hazards were severely exaggerated. It was also pointed out that recombinant DNA experiments not allowed under the current NIH Guidelines are proceeding with the approval of responsible national committees in a number of European countries.

Those concerned that the PRG-RAC were too lax point to the inadequacy of experimental data for a sound evaluation of the potential risks. And they argue that a recombinant DNA experiment permitted under less stringent safety conditions in Europe is irrelevant to the establishment of standards in the United States.

One of the comments at the December 1977 DAC meeting was that "the NIH Guidelines do not adequately deal with the use of recombinant DNA in plants * * *." Other commentators expressed similar sentiments, including the suggestion that "a subcommittee be formed to deal with plants and plant pathogens and make specific recommendations for revision of the Guidelines." In response to this, a Workshop on Risk Assessment of Agricultural Pathogens, composed of distinguished American plant pathologists, was held on March 20-21, 1978 (as announced on March 6 in the FEDERAL REGISTER). Sponsored by the U.S. Department of Agriculture, the National Science Foundation, and the National Institutes of Health, the report of the Workshop is Appendix G to this document. The report was presented to the RAC at its meeting of April 27-28, 1978, and was unanimously endorsed with certain minor amendments. These recommendations, with certain additional minor amendments, have been incorporated into the PRG-NIH.

Two new paragraphs have been inserted at the beginning of Part III of the PRG-NIH. The first reminds the reader to consult Part I, "where a listing is given of prohibited experiments and experiments exempt from these Guidelines." The second is a "general flexibility clause."

Insertion of the latter passage was recommended by the RAC at its April 27-28 meeting. It recognizes that the classification of experiments given in Part III will necessarily be imperfect as investigators in the future devise ways to conduct recombinant DNA research which are not currently foreseen and therefore not explicitly considered in the Guidelines. Also, new data may become available showing that certain experiments are clearly

more (or less) safe than seen at this time and that the currently assigned containment level should be changed. Therefore the inserted passage states that "changes in these levels for specific experiments (or the assignment of levels to experiments not explicitly considered in this section) may be expressly approved by the Director, NIH, on the recommendation of the Recombinant DNA Advisory Committee."

Permissible experiments using E. Coli *K-12 host-vector systems*

Eukaryotic DNA Sources. There was disagreement over those provisions in the Guidelines that allow the principal investigator to choose between two combinations of containment procedures. In several instances, for example, one is permitted to use P2+EK1 or P1+EK2.

This flexibility provision was endorsed by some commentators but questioned by others. It was discussed above in Part II. This concept of investigator flexibility is not a new one; it was allowed under the original Guidelines. Based upon events of the past two years, the RAC merely proposed that the principle be extended to certain specified additional cases where they believe it appropriate. Included in the PRG-NIH are all such specific cases of flexibility recommended in the PRG-RAC.

On the other hand, in certain other specific cases (e.g., DNA from birds), the PRG-RAC recommended that the containment level be P2+EK2, without the option of P3+EK1. Certain commentators urged that in all cases where the containment level of P2+EK2 is given, the option of P3+EK1 be allowed. However, the RAC felt that in view of their increased confidence in the biological containment offered by the EK2 system, P2+EK2 offers more containment than P3+EK1, and that P2+EK2 without the option of P3+EK1 should be the containment level for certain specified classes of experiments. Therefore, there is specified in the PRG-NIH the containment levels of P2+EK2 without the option of P3+EK1 in every case where it appeared in the PRG-RAC.

Discussed below and in the accompanying "Decision" document is the reassessment which was made of the cloning of viral DNA into *E. coli* K-12 at the Ascot Workshop and the April 6-7, 1978, Working Group meeting that endorsed the Ascot report. The RAC at its April 27-28 meeting unanimously endorsed the Working Group report recommending lower containment levels for deliberate cloning of viral DNA into *E. coli* K-12. One of the reasons given originally for the higher containment level for shotgun experiments involving primate DNA into *E. coli* K-12 was the possible *inadvertant* cloning of viral DNA. In view of their recommendation of lower containment for *deliberate* cloning of viral DNA into *E. coli* K-12, the RAC on April 27-28, 1978, reconsidered primate shotgun levels and voted unanimously for new language as follows: *"Primates.* P2 physical containment + an EK2 host-vector. Any lowering of containment below these levels (i.e., for purified DNA or characterized clones) cannot be made solely by an institutional biosafety committee but requires NIH approval." This new language is inserted in the PRG-NIH, as well as a similar lowering of containment for shotgun cloning of cold-blooded vertebrate DNA into *E. coli* K-12.

Prokaryotic DNA Sources. In the 1976 guidelines the section dealing with shotgun experiments in which prokaryotic DNA is inserted into *E. coli* K-12 was subdivided into two parts—"Prokaryotes That Exchange Genetic Information with *E. coli*" and "Prokaryotes That Do Not Exchange Genetic Information with *E. coli.*" In the PRG-RAC it was assumed that all prokaryotes that exchange genetic information with *E. coli* would be exempt from the guidelines by appearing on the "list of non-novel exchangers." Therefore, the PRG-RAC the section dealing with these experiments actually considered only prokaryotes that did not exchange genetic information with *E. coli.* The problem with this approach was discussed by commentators, focusing especially on the case of *Agrobacterium tumefaciens.* It meant that a prokaryote which exchanges genetic information with *E. coli,* and was therefore properly assigned a low containment level under the 1976 guidelines, would under the PRG-RAC either appear on the list and be exempt from the guidelines or, if not appearing on the list for some reason, would require in some cases a higher containment. This was not the intent of the RAC. Therefore, the RAC agreed at their April 27-28 meeting that language should be reinserted into the PRG-NIH covering prokaryotes that exchange genetic information with *E. coli* but do not appear on the list, and this has now been done.

For prokaryotes that do not exchange genetic information with *E. coli* the PRG-RAC proposed that P1+EK2 or P2+EK1 conditions apply only in cases of extensive characterization and RAC approval. A number of commentators objected. Some felt that experiments involving nonpathogenic prokaryotes should be conducted at these lower levels without extensive characterization or RAC approval, and others argued that plant pathogens should not be included with CDC class 2 agents as requiring P3+EK2 containment. The RAC at their April meeting agreed with the commentators. Accordingly, this section of the PRG-NIH has been rewritten.

The EMBO Standing Advisory Committee on Recombinant DNA Research recommends that the containment level for all experiments involving the insertion of novel nonpathogenic prokaryotic DNA into *E. coli* K-12 be P1+EK1. Acting conservatively, the Director has retained in the PRG-NIH the levels of P2+EK1 or EK2 for nonpathogenic prokaryotes that do not exchange genetic information with *E. coli.*

The PRG-RAC received substantial criticisms for identifying all agents classified as class 2 in the CDC's publication "Classification of Etiologic Agents on the Basis of Hazard" (fourth edition, July 1974) as being pathogenic for the purpose of assigning containment levels. Several commentators stated that many of the organisms so classified were harmless and that others were of such low pathogenicity that severe safety precautions were unwarranted. It was also pointed out that the pathogenicity of an intact microorganism and the conjectural hazard of a piece of DNA from such an organism with *E. coli* K-12 were quite different matters. The suggestion of these commentators has been accepted, and thus footnote 1 has been added to the PRG-NIH. This gives NIH the authority, upon the recommendation of the RAC, to consider certain class 2 agents as class 1 for the purpose of these guidelines.

Plasmid, Phage, and Virus DNA Sources. Many of the commentators agreed that both the original guidelines and the PRG-RAC were overly stringent with regard to virus experiments. In commenting on the PRG-RAC, the EMBO Standing Advisory Committee on Recombinant DNA Research wrote, "The EMBO Committee believes that the containment categorization of experiments with animal virus DNA's which is proposed by the NIH Advisory Committee is too indiscriminate and excessively stringent considering the proposed classification of experiments with other classes of DNA and the longstanding, accepted safety precautions for handling intact virus particles and viral nucleic acids * * *." The EMBO Committee proposed (1) that experiments with viral DNA be considered on a case-by-case basis or (2) that a detailed set of recommended categories for such experiments be produced.

A joint United States-EMBO Workship To Assess Risks for Recombinant DNA Experiments Involving the Genomes of Animal, Plant, and Insert Viruses was held in Ascot, England, on January 26-28, 1978. The workship

was attended by 27 distinguished virologists and other scientists from the United States, the United Kingdom, West Germany, Finland, France, Sweden, and Switzerland. A report was published in the FEDERAL REGISTER on March 31, 1978 (43 FR 13748) and constitutes appendix E to the present document. The "Ascot Workshop" concluded:

> The probability that K12 organisms carrying viral DNA inserts could represent a significant hazard to the community is so small as to be of no practical consequence * * *. Viral genomes or fragments thereof, cloned in *E. coli* K12 using approved plasmid or phage vectors pose no more risk than work with the infectious virus or its nucleic acid and in most, if not all cases, clearly present less risk. In fact, the workshop participants agreed that cloning of viral DNA in *E. coli* K12 may provide a unique opportunity to study with greatly reduced risks the biology of extremely pathogenic and virulent viruses.

On April 6-7, 1978 (as announced in the Mar. 17 FEDERAL REGISTER), an RAC-sponsored working group composed of distinguished American microbiologists met to review the report of the Ascot Workshop. The report of this working group is appendix F. The group unanimously endorsed the Ascot report with certain minor amendments. Their report was presented to the RAC, was unanimously accepted, and has been substantially adopted in part III of the PRG-NIH.

Characterized and Purified Clones. Concern was expressed by several commentators about the revisions in the PRG-RAC which would allow the local IBC (with notification to be sent to NIH) to reduce either the biological or physical containment level by one step if (1) the DNA is 99-percent purified and shown to be free of harmful genes before its insertion into a recombinant molecules or (2) the clone replicating the DNA is rigorously characterized and free of harmful genes. In the original guidelines, the reduction in case (2) could only be done with prior NIH approval.

There was support from several commentators for the changes in this subsection. The rationale is explained in the PRG-RAC and the PRG-NIH:

> Many of the risks which might conceivably arise from some types of recombinant DNA experiments, particularly shotgun experiments, would result from the inadvertent cloning of a harmful sequence. Therefore, in cases where the risk of inadvertently cloning the "wrong" DNA is reduced by prior enrichment for the desired piece, or in which a clone, made from a random assortment of DNA's, has been purified and the absence of harmful sequences established, the containment conditions for further work may be reduced.

Some commentators noted the ambiguity and difficulty attendant in the phrase "free of harmful genes." The aforementioned EMBO Committee reports that "several national guidelines for recombinant DNA research state that containment measures may be relaxed once a cloned DNA fragment has been biochemically characterized and shown to be free of harmful genes (NIH guidelines) or devoid of any known pathogenic characteristic (French guidelines). The EMBO Committee believes the latter to be a more feasible requirement, but neither can readily be met, and the committee finds it difficult to suggest what sorts of experimental tests might be devised to meet these requirements."

The terms "characterized" and "free of harmful genes" are unavoidably vague. However, footnote 3 of the PRG-NIH goes on to list five types of data which should be considered in making this determination.

Some commentators were also concerned that this granting of additional authority to the local IBCs for single-step lowering in containment levels might introduce variability in the application of the guidelines. NIH, having considered that possibility, has decided that the principle of promoting local involvement in the implementation of the guidelines outweighs the difficulties that may be encountered in this process. In an effort to minimize these problems, NIH has (1) attempted to make all parts of the guidelines as clear, specific, and unambiguous as possible and (2) expanded the "roles and responsibilities" section to outline functions and responsibilities in greater detail.

Also, the guidelines require that the Office of Recombinant DNA Activities at the NIH be notified in writing of such an action. A mechanism is therefore in place to insure that such actions proceed with an acceptable degree of uniformity.

The question was raised whether a clone of which the containment level was lowered by the IBC at Institution X may, after shipment to Institution Y, be used at the lower level without review by Y's IBC. It has clearly been the intention of both the RAC and NIH that the IBC at the receiving institution must approve the reduction in containment for the handling of the clone in such a situation. The investigator at the receiving institution, however, must handle the clone at the higher level until such permission is granted.

One commentator urged that prior cloning be accepted as a technique for the purification of DNA molecules before their reinsertion into a recombinant molecule. The PRG-RAC specified that purification must be achieved "by physical or chemical techniques." The criterion for the single-step reduction in containment levels in this situation is that the DNA preparation be 99-percent pure. There is no reason, the commentator held, to restrict the means by which such purification is attained. This suggestion has been accepted. The words "by physical and chemical techniques" following the work "purified" have been stricken from the PRG-NIH, better serving the needs of the investigator without reducing the margin of safety to the public and the environment.

One commentator noted that the PRG-RAC might be interpreted as allowing a single-step reduction in containment levels for purification of the DNA before its insertion into a recombinant molecule, and a further single-step reduction in containment once the same molecule has been cloned. This was not intended. Therefore, clarifying language has been added in the PRG-NIH stating that an IBC "may give approval for a single-step reduction in physical or biological containment on receipt of evidence of characterization of a clone *derived from a shotgun experiment* * * *."

Permissable Experiments With Eukaryotic Host-Vectors

Viral Vectors. A number of commentators felt that the stringent containment conditions required, both in the original guidelines and in the PRG-RAC, for introduction of recombinant DNA into tissue culture cells, using viruses as vectors, were unwarranted. The EMBO Standing Advisory Committee on Recombinant DNA Research wrote:

> In experiments involving the introduction of foreign DNA into cultured cells of animals using DNA viruses as vectors, biological containment is assured by the very restricted permissive conditions for the host cells; the only routes by which the recombinant molecule might escape are by chance infection of a contaminating microorganism or within a viral capsid and the size of the recombinant molecule may well preclude its encapsidation * * *. For example, cloning of mouse DNA using polyoma virus as a vector and mouse cells as host should not require precautions more stringent than those routinely used for many years in laboratories studying polyoma virus infection of mouse cells and mice. The EMBO Committee finds the proposals for this class of experiments in the revised NIH Guidelines not sufficiently discriminating because they would impose unnecessarily high levels of physical containment for experiments with many eukaryotic DNA's.

Discussed earlier within the present document was the ascot Workshop report (appendix E) and the report of the Working Group that met on April 6-7, 1978 (Appendix F). The recommendations of the Working Group have been accepted and incorporated into the PRG-NIH.

Plant Host-Vector Systems. Discussed earlier was the Workshop on Risk Assessment of Agricultural Pathogens, held on March 20-21, 1978, under the sponsorship of USDA, NSF,

and NIH. This section of the PRG-NIH has been rewritten on the basis of the Workshop report (see Appendix G of the present document).

Fungal or Similar Lower Eukaryotic Host-Vector Systems. Both the 1976 Guidelines and the PRG-RAC used the same short paragraph for this section, giving little detail, because they noted "the development of these host-vectors is presently in the speculative stage." Since that time a specific host-vector system of this class has been developed—namely, *Saccharomyces cerevisiae* (baker's yeast)—and other similar systems may soon be proposed. Accordingly, this section of the PRG-NIH has been expanded to give more specific instructions on appropriate containment levels.

Synthetic DNA

Because synthetic DNA is now explicitly included in the PRG-NIH (as discussed in Part I of this document), it was necessary to add language to Part III of the PRG-NIH detailing the appropriate containment levels for these experiments. The RAC at its meeting on April 27-28, 1978, approved such language, and it has been inserted in the PRG-NIH.

Proposed Action: Environmental Impact Assessment

Discussed in the Director's "Decision" accompanying the original Guidelines and in the Environmental Impact Statement on their release are—

- Tthe containment safeguards, physical and biological, that protect the laboratory worker, the general public, and the environment;
- The criteria for assessing the possible dangers from experiments involving recombinant DNA molecules; and
- The criteria for matching the assessed possible dangers of individual experiments with the appropriate safeguards.

It was these criteria for the selection of safeguards that guided the deliberations of the Recombinant Advisory Committee in proposing physical and biological containment levels for certain classes of experiments. These criteria were also the basis for recommendations by the scientific work groups on plants and viruses upon which the RAC made further recommendations in April 1978. The basic structure of classification for permissible experiments is maintained throughout the PRG-NIH.

That structure is based on the host-vector system and the source of the DNA. The *E. coli* K-12 host-vector system is considered first, then other prokaryotic host-vector systems, then eukaryotic host-vector systems. To assist the reader in comprehending the structure of the guidelines for permissible experiements, a table is provided outlining the containment levels given in the current guidelines, the PRG-RAC, and the PRG-NIH (see App. A). To assist further in the consideration of experiments under the guidelines, NIH reviewed all experiments supported by NIH as of December 15, 1977, and characterized them in a comparable table (see App. B). This shows the containment levels required for these experiments under the current guidelines, the PRG-RAC, and the PRG-NIH.

The major areas where changes have occurred in the PRG-NIH include the five categories of exempt experiments and those other classes of experiments for which containment levels are lowered. Many of the experiments under the current guidelines would be exempt under the "Exemptions" section of the PRG-NIH, including those in which recombinant DNA molecules are not in organisms or viruses, are from a single nonchromosomal or viral source, or are from species that exchange DNA by known physiological processes. These exemptions are proposed because evidence has led to the conclusion that the experiments pose no significant risk to health or the environment.

Permissible experiments involving *E. coli* K-12 as a host-vector system in the PRG-NIH may generally be done at lower levels of physical and biological containment. A basis for this is the abundant scientific evidence that *E. coli* K-12 cannot be transformed into a pathogen. (See Pt. III of this document for a summary of the scientific information on the safety of this host-vector system.)

Another reason for reducing containment levels when eukaryotic DNA is inserted "shotgun" into *E. coli* K-12 is new knowledge obtained only recently concerning the significant difference between prokaryotes and eukaryotes in the way proteins are synthesized. This newly discovered phenomenon of "intervening," or "spacer," sequences in eukaryotic DNA (1) is discussed in footnote 13 to the "Introduction and Overview" of the accompanying decision document. It makes the expression of eukaryotic DNA inserted "shotgun" into *E. coli* K-12 using "nonengineered" plasmids less likely than had been postulated 2 years ago before the phenomenon of "intervening" sequences in eukaryotes was discovered.

In the PRG-NIH, containment levels have been significantly reduced for the use of viruses as vectors and as a source of DNA for insertion into *E. coli* K-12. The basis for this was the strong support at the public hearing in December 1977 for a scientific analysis on the use of viruses in these experiments. As a result, a meeting sponsored by NIH and the European Molecular Biology Organization, held in Ascot, England, January 1978, provided a rationale for reconsidering containment levels for recombinant DNA experiments involving viral DNA. On the basis of the NIH/EMBO report and a workshop supported by the NIH, the RAC at its April 1978 meeting recommended a complete revision of the sections of the guidelines dealing with viral DNA that is largely reflected in the PRG-NIH. The bases for these revisions are explained in detail in the reports of the Ascot conference and the NIH working group which appear in Appendices E and F. The Ascot conclusions relating to the insignificance of the hazard associated with viral DNA inserts in *E. coli* K-12 are quoted on page 108.

Few recombinant DNA experiments have been conducted with viral DNA, since the overly stringent containment levels of the current guidelines greatly inhibited their use. Under the PRG-NIH, such work would be carefully monitored to insure that any new information on safety or risk were quickly reviewed and any appropriate amendments to the guidelines were made.

Another major area where containment levels have been reduced involves experiments with plant DNA. At the December public hearing of the Advisory Committee to the Director, NIH, scientists from the agricultural community strongly recommended that the guidelines pertaining to experiments with plants be reviewed. In February, NIH, USDA, and NSF convened a meeting of plant scientists, who made a number of recommendations to the RAC. The RAC's recommendations from its April 1978 meeting are reflected in the PRG-NIH. Few NIH experiments are in this area, and developments will need to be closely monitored by the NSF and USDA to determine what work is being done. Again the recommendations comport with safety requirements to assure no significant risk to health or the environment.

In effect, all of the recommendations for permissible experiments and for those exempt from the guidelines are based on new scientific findings or on reassessment of previous information. Evidence indicates that work should proceed because many recombinant DNA molecules produced in laboratories mimic those already present in nature. The PRG-NIH focus on areas of experimentation that need special attention for the possibility of potential hazard. Work in progress that is expected to yield valuable new information will need to be monitored—for example, experiments in which "engineered" systems should permit intentional expression of ge-

netic functions. the current revisions intend to remove as a focus of attention the type of project that does no more than mimic nature and to permit serious attention to new developments that would further the expression of new genetic functions.

All the accumulated evidence on experiments permitted under the guidelines indicates that the proposed revisions would have no significant environmental impact.

The categorization of experiments is based on several premises, as explained in the 1977 environmental impact statement. Shotgun experiments with DNA from primate sources require containment because they involve genes that might function in humans with untoward effects. Containment levels, however, have been lowered here because the concern about a hazard from pathogenic viral DNA's residing in primate tissue has been largely laid to rest in the viral reports. Alternatives in the use of physical and biological containment are provided in a number of cases on the premise that the greater the containment afforded by the host-vector system, the lower the physical containment needed. Requirements continue to be more stringent when the source of foreign DNA is known to be pathogenic or toxigenic, or might be infected with a pathogen, or is known to make harmful products.

For shotgun experiments when the source of DNA is prokaryotic organism, the guidelines specify containment levels according to whether the organism is known to recombine genetic information with *E. coli* in nature. Many of the experiments involving exchangers are now exempt under Exemptions I-E-4 in the PRG-NIH. The lowering of containment recommendations for those experiments in which the source is a prokaryote that does not naturally exchange genetic material with *E. coli* reflects the safety in the use of *E. coli* K-12. Scientific information over the past 2 years shows that recombinant DNA experiments are most unlikely to create new genetic combinations never tested by nature and that the possibility of transforming *E. coli* K-12 into an epidemic pathogen is virtually nil.

In the case of a clone that has been rigorously characterized and is free from harmful genes, the safety is such as to permit provision for actions by the local biohazard committee rather than NIH. Purification greatly reduces the potential for growth of a hazardous organism, and the containment requirements should be correspondingly lower.

The changes in the eukaryotic host-vector systems reflect in large part the recommendations concerning work with viruses and plants. As noted in the original EIS, recombinant DNA experiments here involve the use of systems in which the host cells have little or no chance of escaping from the laboratory as an *E. coli* cell might.

New scientific information indicates that a variety of organisms, such as the lower eukaryotes fungi and yeast, may be useful hosts for experiments with recombinant DNA's; and useful vectors are now becoming available for these systems. Hence, this section of the guidelines has been expanded to detail safe use of these systems. In addition, because of the ability to use synthetic DNA in recombinant DNA experiments, a new section has been added to the PRG-NIH to specify safe containment levels for this research.

NIH has been mindful of the concerns of those who requested that the EIS on the original guidelines contain further information on individual experiments. We have tried to meet that need by the analysis provided in this section and in Appendix B.

At the public hearing on the PRG-RAC in December 1977, some critical comments were directed at NIH's EIS on the original guidelines. Most of the comments centered on NIH policies vis-a-vis permissible experiments. An analysis of those comments appears as Appendix C.

Because of the critical importance of *E. coli* K-12 in recombinant DNA research, an assessment of the use of this organism in recombinant DNA experiments follows.

Background on the use of E. coli K-12 in recombinant DNA experiments

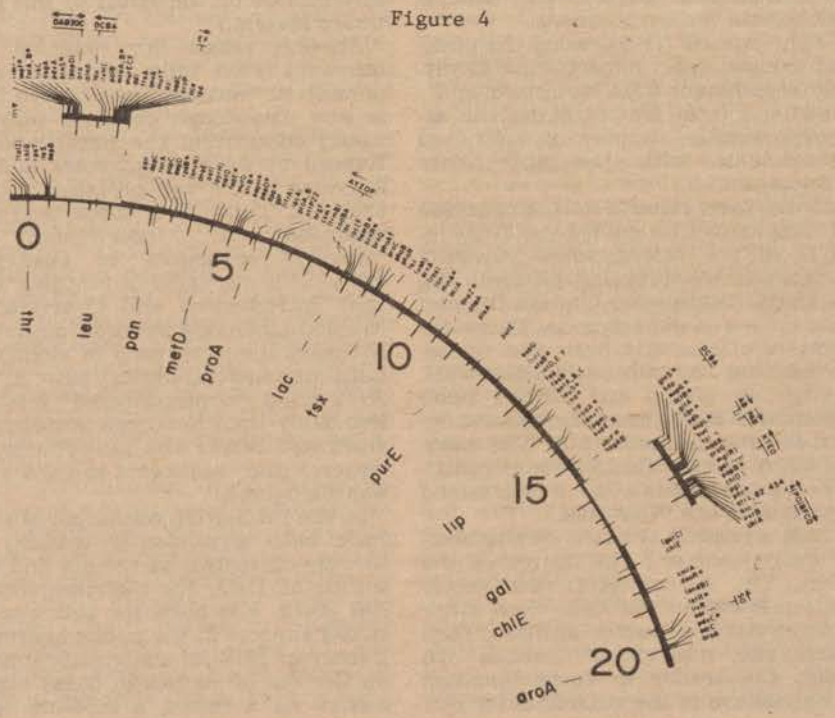
Escherichia coli designates a range of bacterial strains. Each is adapted to live in a certain habitat. Its habitats are found primarily in the vertebrate gut, and it cannot long survive elsewhere—for example, in sewage.(*2*) Some strains are pathogenic, causing disease in the gastrointestinal tract of man or other animals. (*3,4*) One strain of *E. coli*, called "K-12," has been used in laboratory experiments for over 50 years. (*2*) It is not known to have ever caused disease.(*3*)

K-12 became the favorite organism for genetic research because it reproduces rapidly and thrives under controlled laboratory conditions. No living creature is known more thoroughly. Its single chromosome can be easily manipulated by genetic means, permitting its gene structure to be mapped. This work has greatly advanced understanding of how genes express and regulate inherited characteristics.

The chromosome of K-12 is a circular molecule of DNA with about 4 million subunits. These compose 3,000 or 4,000 genes, of which about 650 have been identified and assigned locations. (*5*) An arc of the genetic map of *E. coli* K-12 is shown below.(*5*)

The NIH guidelines limit the vast majority of recombinant DNA experiments to the use of *E. coli* K-12 as host for the foreign genes. This is because the unaltered organism is nonpathogenic and well known in its natural properties—both factors lending confidence that it can be handled safely.

Figure 4



There are those, however, who view the ubiquity of *E. coli* in vertebrates as an argument against the use of even the K-12 strain as a host for foreign DNA. Concern has been expressed, for instance, that the bowels of persons on anitbiotics, ill persons, human infants, or members of other species may be susceptible to colonization.(*7*) Moreover, if *E. coli* K-12 can survive in a person or animal, it might confer genetic characteristics to hardier bacterial inhabitants.

Scientists engaged or interested in recombinant DNA research have addressed these concerns in three ways—first, through guidelines specifying practices and conditions of containment for experiments classified according to the presumed hazard; second, through attempts to develop safer hosts and vectors; and third, through risk-assessment studies. The results of a workshop held in June 1977 at Falmouth, Mass., to evaluate the potential risk of recombinant DNA experimentation with *E. coli* K-12 will be discussed below.

The Evidence That E. Coli *K-12 Is Nonpathogenic.* The laboratory variants of K-12 permitted in recombinant DNA experiments have never been reported to cause disease, even in laboratory workers. K-12 has been grown in large quantities—up to hundreds of liters containing as many as a trillion bacteria. These cultures have been produced in countless laboratories the world over, and under containment conditions lower than the minimal ones in the NIH guidelines. K-12 has none of the properties generally associated with pathogenic bacteria.(*8–15*) It does not—

- survive and multiply readily in natural environments.
- spread from animal to animal or plant to plant,
- multiply readily on body surfaces or intestines and lungs,
- penetrate animal cells or spread through animal bodies,
- produce a toxin or otherwise alter other living things to cause disease, or
- resist normal body defense mechanisms.

Even after as many as 10 billion K-12 organisms have been ingested, their multiplication in normal humans is only transient, and after a time none can be recovered.(*10, 11, 12, 14*) Thus, K-12 does not establish itself as a permanent resident of human beings. On the other hand, K-12 can reside under abnormal conditions, as during antibiotic therapy.(*16*)

A micro-organism, in order to cause disease, must have the genetic capability to do so, as well as the ability to establish itself in the body. It is difficult to conceive how K-12, itself nonpathogenic, could become pathogenic as a result of genetic manipulation. Highly attenuated, it is known only to inhabitat the biological laboratory.

Even when genetic determinants of pathogenicity in other *E. coli* strains were introduced into K-12, no instance of capacity to induce diarrheal disease or urinary tract infection could be detected.(*14, 15*) The workers conclude that the inadvertent transformation of K-12 into a highly pathogenic form by the introduction of a single fragment of foreign DNA is highly unlikely.(*15*) Indeed, the number of characteristics that a microbe must have in order to cause disease is believed to be great, not to mention additional characteristics needed to produce an epidemic.(*17*)

Transfer of Foreign DNA from E. coli *K-12.* While it would appear impossible to render *E. coli* K-12 pathogenic by the introduction of foreign DNA, there is still to be considered whether the inserted fragment could be transmitted to another bacterium with which the K-12 comes in contact, including other strains of *E. coli.* Such a transmission might convert the recipient into a pathogen or render a pathogen more viable. The case of plasmid vectors is considered first.

Plasmids are intracellular particles composed of DNA and not dependent on chromosomes for their replication. Hence, they can be used as vectors, or vehicles, for transporting foreign DNA into the bacterial host, where they multiply and propagate the genes they bear. Certain plasmids (called "conjugative") are inherently able to migrate from one bacterial cell to another. These are prohibited for nearly all recombinant DNA experiments. Only plasmids not capable or barely capable of spontaneous intercellular migration ("nonconjugative") may be used.

The nonconjugative plasmid's ability to migrate is augmented if the cell harboring it is invaded by a conjugative plasmid, which may confer this property. Then even the non- conjugative plasmid may become a potential DNA-bearing invader. It has been calculated, however, that the chance of this occurring with certain K-12 plasmid systems is less than 1 in 10^{16} (10 quadrillion) K-12's surviving per day in the intestine of warm-blooded animals.(*13, 14*) The probability is even lower in sewers, sewage treatment plants, and waterways. It should be noted that since most of the estimates of probability are based on data obtained under laboratory conditions, animal and human feeding studies are needed to verify the predictions.(*18*)

Consideration must also be given to the question of transfer of foreign DNA from the initial K-12 host to other bacteria by means of bacteriophage vectors. Bacteriophages are viruses that infect only bacteria. They could escape the laboratory either as mature infectious particles or in bacterial hosts in which the phage DNA is carried as a plasmid or within the DNA of the cell.

The survival of phage DNA when released as infectious particles depends on their stability in nature, their infectivity, and the probability of effective encounters with naturally occurring *E. coli.* The bacteriophage used in recombinant DNA experiments is known as *lambda.* It is considered very unlikely to survive and to infect resident *E. coli* in animals and humans, being highly sensitive to stomach acid, reluctant to infect smooth *E. coli* cells (the type normally found in the gut), and susceptible to drying, as would occur if it escaped into the air. Moreover, *E. coli* vulnerable to lambda is uncommon in nature. Infective lambda ingested in large amounts (10^{11}, or 100 billion, particles) could not be detected in human feces.(*19*)

Establishement of lambda as a resident of the *E. coli* host cell's DNA is a well-known example of natural recombination, In certain cases, it is a frequent event, as likely to occur as not. Hence, this mode of escape would be the preponderant laboratory hazard. However, most variants of lambda used (or under consideration for use) in recombinant DNA experiments have a much reduced ability to become so incorporated.(*20–22*) Here the probability drops to 10^{-5} or 10^{-6}—1 in 100,000 or 1,000,000.(*23–25*)

The estimates for containment in the use of bacteriophage host-vectors, while not exact, are sufficient to assure that the probability of transferring a foreign DNA fragment from the original K-12 host to other bacteria is remote.

Ability of E. Coli *K-12 To Survive and Spread in Nature.* Thus far, the suitability of K-12 for recombinant DNA experiments has been considered in relation to its ability to do harm

either directly or through transfer of a foreign DNA fragment to another bacterial cell. These properties will depend on the ability of the K-12 to survive, multiply, and infect other living organisms. As already described, K-12 is poorly equipped to survive in natural environments; but if it should survive and multiply, it is still unlikely to infect living things. *E. coli* are seldom spread by aerosols; they are primarily spread by ingestion of contaminated food and water. Between 10^6 and 10^9 (1 million to 1 billion) cells of pathogenic *E. coli* are required to cause disease (*12,25*). In other words, at least a million bacteria would be required to cause disease in a single person if some K-12 did become pathogenic.

The guidelines emphasize protection of laboratory workers because they are the persons most at risk. They are also the most likely means by which recombinant DNA might be spread. Should a worker carry such agents out of the laboratory, however, the probability that others would be affected is still very low, and the risk of a resulting epidemic is virtually nonexistent. There is abundant evidence for this assertion. It has long been known that the separation of sewage from food and water supplies prevents epidemcis of enteric bacteria such as *E. coli*.

The following excerpt from a letter by Roy Curtiss III to Donald S., Fredrickson discusses the K-12 strain of *E. coli* in relation to infectivity.(*13*)

In terms of communicability of *E. coli* K-12, we know that enteric diseases caused by enteropathogenic *E. coli* and various strains of Shigella, Salmonella and Vibrio are transmitted by contaminated food and water and that manifestation of disease symptoms requires consumption of approximately 1 million bacteria. Such enteric diseases are seldom spread by aerosols. Indeed, it is well known, for example, that cages of mice infected with Samonella can be housed in the same room with uninfected mice which remain uninfected. The finding that *E. coli* cells can be recovered from the nasopharynx of approximately 5 percent of those humans tested might suggest that aerosol spread could occur. Such *E. coli* cells, however, are only intermittently present in the nasopharynx and are usually found at concentrations too low to initiate an infection even if they were representative of a pathogenic strain. They most likely get into the nasopharynx due to poor personal hygiene. After learning of these observations quite some years ago, I monitored my nostrils and skin for the presence of those *E. coli* K-12 strains I was working with. I was successful in detecting these strains about 10 percent of the time when the monitoring was done at the end of the work day, but never obtained positive results when the monitoring was done the next morning. I should hasten to add that my research with *E. coli* K-12 at that time involved mouth pipetting and other aerosol-generating procedures on an open lab bench: procedures and conditions which are not permitted by the NIH Guidelines. These results, preliminary as they are, nevertheless suggest that *E. coli* K-12 does not colonize the nasopharynx. Based on these observations, the fact that *E. coli's* normal ecological niche is the colon and the fact that transmission of enteric diseases is by ingestion of contaminated water and food, I doubt that *E. coli* K-12 could be converted to an air-borne "infectious" agent by introduction of recombinant DNA. In terms of the more usual means for spread of enteric pathogens, it is evident tha enteric diseases are bery well controlled in the United States by sanitary engineering, even though there have been reports of poor water quality in some parts of the country and higher-than-desired levels of pollution of rivers, streams, etc. There is however, a concerted effort to improve biological waste water treatment and thus lessen pollution and improve water quality. Even if there were a natural catastrophe such as caused by an earthquake, tornado, hurricane, etc., it is unlikely that *E. coli* K-12 containing recombinant DNA could initiate or sustain an epidemic in view of K-12's inability to colonize and overcome host defense mechanisms.

Seeking a consensus on the matter of risk assessment in recombinant DNA research, with particular reference to the use of *E. coli*, the National Institutes of Health sponsored a workshop in Falmouth, Mass., on June 20-21, 1977. In attendance were approximately 50 invited participants and observers, from the United States and abroad, including experts on all aspects of infectious disease. The following excerpt from a letter by the workshop chariman, Sherwood L. Gorbach, to Donald S. Fredrickson summarizes the principal conclusion:

CONSENSUS AGREEMENT

An important consensus was arrived at by the assembled group which I felt was of sufficient interest to be brought directly to your attention. The participants arrived at unanimous agreement that *E. coli* K-12 cannot be converted into an epidemic pathogen by laboratory manipulations with DNA inserts. On the basis of extensive studies already completed, it appears that *E. coli* K-12 does not implant in the intestinal tract of man. There is no evidence that non-transmissible plasmids can be spread from *E. coli* K-12 to other host bacteria within the gut. Finally, extensive studies in the laboratory to induce virulence in *E. coli* K-12 by insertion of known plasmids and chromosomal segments coding for virulence factors, using standard bacterial genetic techniques, have proven unsuccessful in producing a fully pathogenic strain. As a result of these discussions, it was believed that the proposed hazards concerning *E. coli* K-12 as an epidemic pathogen have been overstated. Such concerns are not compatible with the extensive scientific evidence that has already been accumulated, all of which provides assurance that *E. coli* K-12 is inherently enfeebled and not capable of pathogenic transformation by DNA insertions.

The entire letter from Gorbach is quoted in the NIH environmental impact statement, part II, appendix M.(*27*) The proceedings of the Falmouth workshop on risk-assessment have been published in the May 1978 Journal of Infectious Diseases.

There remains the question whether the insertion of a foreign DNA fragment into K-12 will significantly alter the properties of the latter with regard to survival and multiplication, or the ability of the plasmid and bacteriophage vectors to be spread. The improbability of converting K-12 to a pathogen has already been discussed. Changes in ability to survive and multiply would be expected to involve not only the changes in the K-12 itself, or the plasmid or bacteriophage, but also the nature of the environment in which it finds itself. The subject is discussed in the section of this document entitled "Risk and Benefits of Recombinant DNA Research."

Attenuated K-12 Systems. Theoretically, the most desirable bacterial recipient of recombinant DNA would be a species uniquely adapted to carefully controlled laboratory conditions and unable to survive or transmit DNA to other organisms in any natural environment. This means that it should be unable to establish itself as a long-lived and multiplying resident in or on living things, or in soil or water. In addition, these properties should not be significantly altered by recombination of the bacterium's DNA. The organism should also, of course, lend itself to manipulation for successful execution of experiments.

No bacterium meeting all these requirements is known. It is possible that no such creature exists in nature. Available bacterial systems must be evaluated for relative safety and utility, depending on the extent to which they approach the ideal. The foregoing summary of knowledge concerning K-12 and its known plasmids and bacteriophages indicates that these systems measure up well with the ideal criteria, and can therefore be recommended for use in recombinant DNA research.

The K-12 systems, extant and projected, are known as EK1, EK2, and EK3, referring to increasing degrees of attenuation. The guidelines permit the use of EK1 for those experiments whose potential for hazard is regarded as nil, low, or minimal. For experiments judged to have a somewhat higher (though still conjectural) potential for hazard, the guidelines require the further attenuated system EK2. Here, properties of the K-12 and the vectors must be so modified as to minimize the chance of the vector surviving in its host outside the laboratory and migrating to other hosts. EK3 systems are even stricter, requiring, for example, the use of vectors that cannot propagate outside the host. So far, no EK3 systems have been certified. [In the proposed revised Guidelines (PRG-NIH), the EK

systems are retained within the broader host-vector systems (HV) classification, providing more specificity.]

Implications of the use of EK2 containment are elucidated in the following passage from a paper presented by Bernard D. Davis at a forum on recombinant DNA held by the National Academy of Sciences, March 7-9, 1977.(*2*)

A very large safety factor is added by the provision in the present guidelines for biological containment. All work with mammalian DNA must be carried out in EK2 strains, which have a drastically impaired ability to multiply, or to transfer their plasmid, except under very special conditions provided in the laboratory. The presently certified EK2 strain has several stable mutational defects (i.e., deletions) that prevent it from multiplying under the nutritional conditions of the gut. But the protection goes much further, and reaches a degree that is unprecedented in the annals of man's exploration of potentially hazardous new materials: this material has been coded for self-destruction. For example, these mutant cells require diaminopimelate, a constituent of cell wall; and without it they can continue to grow and expand but cannot form more wall, and so they quickly burst. Accordingly, under conditions similar to those in the gut such an EK2 strain not only fails to multiply, but less than 1 in 10^8 cells survives after 24 hours—and it would be an extraordinarily sloppy laboratory accident that would result in ingestion of as many as 10^8 cells. In addition, while the cells are dying off in the absence of diaminopimelate they are severely impaired in their ability to transfer plasmids to other, well-adapted cells—and this is the important point for the danger of spreading harmful genes. Finally, not only the cells but also the plasmids being used to carry recombinant genes are also weakened mutant derivaties, selected for severe impairment of their ability to be transmitted from the host cell to another cell.

We thus see that, even with a strain known to carry the gene for a potent toxin, the production of disease in a laboratory worker would require the compounding of two low probabilities: that the strain will initiate an infection and that it will survive long enough to cause harm despite its several disadvantages—that of being a laboratory-adapted strain, that of carrying the burden of foreign DNA, and that of carrying the very large burden of being a suicidal EK2 strain.

The criteria for NIH certification of an EK2 system have been defined and enlarged during the past year. Extensive data are required and very demanding standards have been set. Such organisms are being designed and constructed by NIH contractors and other interested investigators. Their use in recombinant DNA experiments is not allowed until they have been certified by the Director, NIH, upon recommendation by the Recombinant Advisory Committee. The NIH environmental impact statement describes the criteria for certification and lists the certified EK2 systems as of July 1977.(*28*) It should be noted that the same depth of experience with K-12 that recommends its utility as a host for recombinant DNA experiments is central to the ability to manipulate it for the purpose of improving its safety.

An important recent paper was published by two British workers, Petrocheilou and Richmond on the absence of plasmid or *E. coli* K-12 infection among laboratory personnel.(*29*) In testimony before the Subcommittee on Science, Technology, and Space of the Senate Committee on Commerce, Science, and Transportation, on November 10, 1977, Dr. Oliver Smithies, professor of medical genetics and genetics at the University of Wisconsin, interpreted the Petrocheilou and Richmond results as follows:

Twice weekly for over 2 years these workers tested the feces of five laboratory persons who had been using without special precautions the laboratory strain of *E. coli* called K-12, together with a transmissible plasmid. Neither the *E. coli* K-12 nor the transmissible plasmid was ever found in the feces during these tests. (Transmissible plasmids are naturally occurring circular pieces of DNA that can replicate inside bacteria and which, in nature, transfer genes between them.) So, with *E. Coli* K-12 and a transmissible plasmid, the risk of the plasmid or its host K-12 getting into the feces and surviving to any appreciable extent is less than one per laboratory worker per 10 years of lab work, even when no special precautions are taken.

Now, under the NIH guidelines, none of the even conceivably hazardous experiments are performed in this type of *E. coli*, K-12. Such experiments require a specially weakened strain, Chi 1776, which introduces a safety factor for survival of greater than 100 million. Chi 1776 has been proven by tests to survive 100 million times less well than K-12.

In addition, such experiments require the use of a nontransmissible plasmid which introduces a safety factor for transfer of the plasmid to other bacteria of about 100 million.

Let me emphasize again that this type of work requires a nontransmissible plasmid; that is, a plasmid derived from a transmissible plasmid by eliminating the mechanisms for transfer of the plasmid between bacteria.

So the risk of Chi 1776 strain of *E. coli* K-12 surviving in the feces or of the recombinant DNA plasmid being transferred to some other bacteria becomes less than one chance per 100,000 laboratory workers working for 10,000 years without special physical precautions.

This is what is meant by a "negligible risk."

When we consider that the guidelines require also very special physical precautions, you can see why I think the risk is no longer worth considering.

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IV. Roles and Responsibilities

Analysis of Current Guidelines

The Guidelines contain a large section, Part IV, defining the roles and responsibilities of individuals and institutions in assuring compliance with required containment levels. The procedures described are primarily directed at grantees of the National Institutes of Health. Similar procedures are in force for work within NIH laboratories and for work sponsored by NIH under contracts.

The principal investigator is required to assess any potential biohazards, to institute appropriate safeguards and procedures, to minimize effects of possible accidents by planning, to train and inform all personnel, and to report any accident or any serious or extended illness of a worker. All of these must be carried out on a continuing basis. Thus, the primary responsibility for conducting experiments according to the Guidelines is in the investigator's hands.

Further, in applying for grants to carry out experiments with recombinant DNA, the investigator must include an estimate of the potential biohazards and a statement of the containment procedures to be used. The application must include certification of the existence and availability of appropriate facilities, procedures, and training. The Guidelines indicate that institutions in which recombinant DNA experiments are carried out must establish biohazards committees that examine equipment and facilities and certify their compliance with the requirements. Such committees will also serve as a source of advice and reference on physical containment facilities, on properties of biological containment, and on training of personnel.

According to the Guidelines, the certification and the investigator's assessment of the hazard and containment would be considered by NIH study sections during the normal scientific review of the application. The Guidelines leave flexible the question of resolving any differences between the investigator's evaluation and that of the study section. The Guidelines do state, however, that if differences cannot be resolved, the matter should be referred to the Recombinant Advisory Committee or the NIH Office of Recombinant DNA Activities.

Application of the guidelines to work not supported by NIH

Several agencies of the U.S. Government other than the National Institutes of Health provide support for biological and medical research. Some of these currently sponsor recombinant DNA experiments, and others may do so in the future. Activities of the research agencies represented by the Federal Interagency Committee on Recombinant DNA Research were reviewed by the Committee in the fall of 1976. All member research agencies adopted the NIH Guidelines and standards, including the National Science Foundation, the Department of Agriculture, the Energy Research and Development Administration, the Department of Defense, the National Aeronautics and Space Administration, and the Veterans Administration.

Several conferences have been held at NIH and at other relevant Government agencies with representatives of private industry in the United States. As best detemined by the Federal agencies, recombinant DNA research conducted in the private sector complies with the physical and biological standards of the NIH Guidelines. Relevant industries have agreed to follow the Guidelines on a voluntary basis.

The issue of recombinant DNA research has been studied by national and international bodies in many countries. In most cases some form of control has been recommended, but nowhere has a total ban on the research been advocated. Canada, the Federal Republic of Germany, France, the Soviet Union, and the United Kingdom have issued guidelines that differ in detail but are similar conceptually to the NIH Guidelines. Other countries are generally following the NIH or U.K. Guidelines, including Denmark, Israel, the Netherlands, Sweden, and Switzerland. The international Council of Scientific Unions and the World Health Organization have urged nations to adopt the principles embodied in these two sets of guidelines. The U.K. Guidelines have been endorsed by the European Science Foundation and the European Molecular Biology Organization.

Scientific and governmental activities comparable to those in the United States have been under way in the United Kingdom since January 1975. A working party established at that time recommended that recombinant DNA research in the United Kingdom be permitted to continue under appropriate controls. In August 1976 a followup working group chaired by Sir Robert Williams issued a report establishing guidelines.

In Canada, in March 1976, a special committee of the Canadian Medical Research Council recommended guidelines to govern the handling of recombinant DNA molecules in Council-supported research. The Council adopted these guidelines in February 1977.

Many other nations have reviewed recombinant DNA activities to determine what measures were necessary for safety. With the urging of regional and international bodies, most have adopted the NIH or U.K. Guidelines as a basic framework for safety practices and procedures.

Alternatives: RAC-Proposed Revisions

Part IV (Roles and Responsibilities) of the PRG-RAC is described below. As in the current (1976) Guidelines,

this part of the PRG-RAC was designed to provide an administrative framework for implementation.

Institution

In the PRG-RAC as compared with the current Guidelines, several changes were proposed in the responsibilities of the institution. Responsibilities that were added or further detailed included: (1) a requirement for insuring the training of research personnel and the use of good microbiological technique, and (2) a requirement to determine the need for medical procedures, with recommendations of possible specific practices.

Institutional biosafety committees

Membership of the IBC's was clarified by a recommendation to include other than scientific members. In the PRG-RAC, institutional biosafety committees (called "biohazards committees" in the current Guidelines) are given the discretion to approve single-step reductions in containment levels for experiments with characterized clones and purified DNA. The IBC's would be required to notify the NIH Office of Recombinant DNA Activities (ORDA) of these approvals.

Biological safety officer

Institutions at which P3 and P4 level recombinant DNA work is conducted would be required to have a biological safety officer, whose specific roles and responsibilities are outlined.

Principal Investigator. The role and responsibilities of the principal investigator would remain basically the same, except for the important addition of a requirement for training in microbiological techniques. Responsibility for the determination of the practices necessary for medical surveillance would be relocated to the institution.

NIH responsibilities

Office of the Director. The responsibilities of the Director remain unchanged. A sentence has been added that clarifies the Director's authority to implement the Guidelines and to be the final arbiter in their interpretation.

Recombinant advisory committee

There were no changes in the current responsibilities of the RAC. There were, however, clarifications of the scope of some duties—for example, the certification process. The language of the 1976 Guidelines caused some confusion about the certification of EK2 (HV2) and EK3 (HV3) host-vector systems. In practice, the certification process, clarified in the PRG-RAC, involves a two-step procedure: (1) The RAC's recommendation to the Director, NIH, that a particular host-vector system be certified; and (2) certification of the system by the Director. The rationale for the procedure is that it allows the Director to solicit the opinions of additional experts before making a financial decision on certification.

The RAC's authority to recommend exceptions from the prohibitions was also clarified. The 1976 version of the Guidelines envisioned the possibility of the RAC's recommending an exception to the 10-liter limit on culture volume for recombinant DNA's known to make harmful products. The proposed revision would extend the possibility of an exception to the five other classes of currently prohibited experiments.

The general rationale for this addition is twofold: the RAC's inability to foresee all possible future circumstances and its desire to specify, within the limits of strict safeguards, the possibility of an exception for compelling social or scientific reasons. A more immediate and specific justification for the paragraph on exceptions from the prohibitions is that the risk-assessment studies necessary for a clearer understanding of the potential biohazards of recombinant DNA research may be technically prohibited by the current Guidelines, unless there is a mechanism for approving exceptions.

Alternatives: Public Commentators

Institutional responsibilities

This section of the Guidelines drew considerable comment directed to the roles and responsibility of the local institution and its several constituents. Generally, commentators requested more information and greater clarification of the structure and operation of the IBC, the function of the biological safety officer, and the duties of the institution. The suggestions and comments were carefully considered, in view of the importance of this section to successful implementation of the Guidelines and therefore safe conduct of the research.

NIH has a special responsibility for leadership in developing and promoting safety programs relevant to recombinant DNA experiments. Accordingly, as in 1976, another committee chaired by Dr. W. Emmett Barkley, Director of the National Cancer Institute's Office of Research Safety, was convened to address concerns raised. As a result, and in response to a number of commentators' requests, the substance of Appendix D has been revised and republished as a supplement to the Guidelines. The revised Guidelines also retain requirements for emergency plans to cover accidents and strengthen the requirement for training of all recombinant DNA researchers in safe laboratory procedures.

The intent of this section, as before, is to integrate safety practice into the conduct of recombinant DNA research and to assign responsibilities for this to the principal investigator, institution, IBC, and biological safety officer. It is important that these responsibilities be stated in an unambiguous manner. In response to many commentators. Part IV has been restructured to present some of these functions in greater detail and clarity. The appendices contain additional complementary information on roles and responsibilities, including material for IBC's and biological safety officers.

Expanded Responsibilities. In response to several comments, the review of research has been broadened in the PRG-NIH to cover all recombinant DNA research at an institution receiving NIH funds for this purpose, whether or not the specific recombinant DNA project is funded by NIH. While this increases the responsibility of the institution and the IBC, it is believed that the overall safety of recombinant DNA research will be enhanced. To reflect more closely the spirit of the Guidelines, the name "institutional biohazards committee" is proposed to be changed to "institutional *biosafety* committee."

Several generic comments deserve to be highlighted, as they represent significantly increased authority to be delegated to the institution. In 1976 the RAC did not accept commentators' suggestions to require local committees to make an independent evaluation of the containment levels required by the Guidelines for individual research projects. It was therefore stated in the 1976 Decision that NIH would not require local institutions to have their committees perform this function, although they would not be prohibited from doing so. Commentators have now noted that an IBC, in order to to accomplish its mandated responsibilities under the 1976 Guidelines, including the review and approval of recombinant DNA research projects, must implicitly determine containment conditions. In order to clarify the committee's role, the assessment of appropriate containment levels is now made an explicit responsibility of the IBC.

In addition, institutions through their biosafety committees would be given increased responsibility for primary overview of this research, as they have been delegated the authority to approve or disapprove proposed recombinant DNA projects. NIH, through ORDA, will conduct a review of institutions' actions, upon registration of the projects, to ensure compliance with the NIH Guidelines, thereby maintaining a national standard. This action has been in response to several comments calling for increased local responsibility and a simpler adminis-

trative process in regard to gaining approval for research to proceed.

In view of the unreliability of Federal surveillance to enforce these standards, it is essential to increase the authority and responsibility of the local institution. It was requested that the IBC's have a role if legislation in this area is adopted. This concept is endorsed by the House Committee on Interstate and Foreign Commerce in its Bill Report of March 24, 1978, on the Recombinant DNA Act:

> It is the view of the committee that the appropriate portions of the administrative requirements of section IV of the NIH Guidelines are a reasonable model upon which the Secretary could base administrative regulations. In particular, the current practice in the NIH Guidelines of delegating to local biohazards committees most of the responsibility for the inspection of facilities and the approval of the specific safety requirements appropriate to each project or activity is an effective and relatively inexpensive administrative mechanism.

A number of recommendations were received regarding the membership of IBCs. In 1976, suggestions were made for broadening IBC representation to cover not only various disciplines related to recombinant DNA technology, safety, and engineering, but also to include members knowledgeable in applicable laws and regulations, standards of practice, community attitudes, and health and environmental considerations. These diverse points of view were either to be included or made available to the committees. The language in the PRG-RAC calls for a diversity of membership, but would not mandate public members. In response to several requests, and in view of increased responsibility at the local level, a provision is included in the PRG-NIH that "no IBC may consist entirely of persons who are officers, employees, or agents of, or are otherwise associated with the institution, apart from their membership on the IBC."

A number of other recommendations were received from public commentators relating to more specific issues concerning the various responsibilities of the institution and its constituents. These recommendations and the PRG-NIH decision are considered below under the appropriate headings.

Institution. A number of points were raised by commentators concerning health monitoring by institutions. NIH was requested to develop a model for institutional medical surveillance for recombinant DNA research workers. An NIH committee is reviewing this area and has made recommendations as to what such a program might include. This proposal, which calls for monitoring illnesses, collecting serum samples, and keeping a register of agents handled, is responsive to several suggestions received on this issue, and has therefore been adopted in the PRG-NIH. Additionally, Appendix D will include more detailed information on medical surveillance.

A collaborative effort has been initiated between NIH and the Center for Disease Control (CDC) to establish a mechanism for providing advice, consultation and, if necessary, assistance regarding major accidents in laboratories conducting recombinant DNA research. It was not considered necessary to have a standing "strike force" as suggested by one commentator; but in the event of an emergency, a team of experts from NIH and CDC could be formed to respond.

The issue of medical monitoring is one of considerable interest to NIH. This is a general problem not unique to DNA research. As one commentator noted, a routine health monitoring and reporting program might well be instituted for personnel engaged in areas of research besides recombinant DNA, such as tumor viruses and pathogenic organisms. The state-of-the-art, however, is primitive in terms of what can be done to monitor workers' health, and particularly in the area of recombinant DNA research, where there is no known hazard.

Grievance procedures for workers under the Guidelines were requested, but this is not considered necessary, as the rules and regulations of the Occupational Safety and Health Act (OSHA) already provide such a mechanism. OSHA standards and procedures apply to most institutions, so it is not considered necessary to require in the Guidelines that IBCs ensure OSHA compliance. Further, the Federal Interagency Committee on Recombinant DNA Research includes the Occupational Safety and Health Administration (Department of Labor), assuring cooperation at the Federal level.

Institutional Biosafety Committee. Several commentators requested more detail on IBC duties. This has been accomplished in "Laboratory Safety Monograph—A Supplement to the NIH Guidelines for Recombinant DNA Research." For example, information is included there on facility certification, periodic inspections, and monitoring.

It was suggested that biosafety committee meetings be open to the public. The Guidelines currently require only that the minutes by publicly available. In view of possible discussion of proprietary information and patent rights, meetings cannot always be open. Local committees, however, should consider having open meetings when possible.

The question was raised concerning conflict of interest of local committee members. Addressing this important point, a provision in the PRG-NIH prohibits an individual from being involved in the review of a recombinant DNA project in which he or she was engaged or had a direct financial interest.

Biological Safety Officer. Since the passage of OSHA, most institutions have established occupational safety and health departments with safety officers. There are no standard certification procedures for such individuals, although their qualifications, in many cases, could be commensurate with those of a biological safety officer. The Laboratory Safety Monograph provides in detail the kinds of qualifications biosafety officers should have. NIH is developing a training course for biological safety officers and other campus safety personnel. Requests for information should be directed to Dr. Emmett Barkley, Office of Research Safety, National Cancer Institute, NIH.

Princial Investigator. Commentators remain concerned about the quality and uniformity of safety training. NIH is responding to this by placing as a high priority the development of training standards and courses. Currently, NIH is supporting a Working Panel of the American Society for Microbiology (ASM) that is considering standards of training in micro- biological techniques for recombi- nant DNA research. When a report is submitted to NIH, it will be shared with institutions, IBC's, and principal investigators for their use. National certification, however, should not be attempted until the ASM-NIH criteria for training have been adopted and evaluated.

It should be noted that, aside from the Nuclear Regulatory Commission's standards for training in radioisotope work, there seem to be no other training criteria at present in biomedical research. Thus, the work of the ASM Panel will establish a precedent. For these reasons NIH should proceed carefully and in stages while promoting safety training for researchers. NIH will develop training courses based on these standards and will make them widely available.

NIH responsibilities

As in the public hearing on the Guidelines as proposed in 1976, many commentators again urge openness, candor, and public participation in the revision process, emphasizing shared responsibility and accountability from the local to the national level.

Due-Process Considerations. A focus of public comment at the December 1977 hearing was on "procedural due process" to ensure public participation in the development of NIH recombinant DNA policies. Much of the public testimony and comment in letters focused on public representation on

committees. Also stressed was the need for public notice of all meetings, and for procedures to ensure public participation in the exercise of responsibilities by the NIH Re- combinant Advisory Committee (RAC), the Office of the NIH Director, and the Advisory Committee to the Director (DAC).

Several commentators specifically urged that the Guidelines spell out procedures—

- To develop and promulgate the list of "non-novel experiments" and to amend the list;
- To certify host-vector systems;
- To permit the Director, on the advice of the RAC, to grant exceptions from prohibited experiments (as for risk-assessment studies), and
- To modify the Guidelines in the future.

There were also suggestions that guidance be given on how to deal with infractions of the guidelines. Specifically, one commentator suggested that procedures should outline in detail –

- How charges of noncompliance could be brought,
- How charges of noncompliance would be evaluated,
- What opportunities would be provided for the principal investigator and his institution to defend themselves against charges, and
- What procedures would be available before the termination of funding or other penalties are invoked.

Because of the RAC's key role in the development and monitoring of NIH recombinant DNA policies, a number of comments were directed to the committee's nature and functions. Many commentators focused on its membership, urging that the guidelines define procedures for the nomination and selection of members. Suggestions for potential membership included more representation for certain scientific disciplines, such as virology and microbiology; greater representation from the occupational and environmental health and safety community; and more public representation, including perhaps a "dissenter" from current NIH policies.

A number of comments concerned RAC operations. The committee was urged to formalize schedules so that all concerned would know when meetings would be held over the next 2 to 3 years. Further, it was urged that notices and complete agendas be placed in the FEDERAL REGISTER for each meeting, that all documents for committee consideration be made available to the public, and that the NIH pay for public witnesses to attend RAC meetings.

In response to these comments, part IV of the PRG NIH has been reorganized extensively. The responsibilities from the local to the national level are more clearly stated and defined. For NIH responsibilities, procedures suggested by commentators have been specified to afford opportunity for public comment. A special appendix to the PRG NIH includes relevant implementation documents from ORDA that explain the administration of the NIH guidelines at the local and national levels.

Part IV of the PRG NIH has more clearly defined a structure for responsibilities at those levels, with opportunity for public and scientific participation. It formalizes a process that has been occurring informally. Flexibility, however, remains essential to avoid unnecesary and protracted delays in decisionmaking. Clearly, a full panoply of clearance procedures, including a public hearing, is not essential for most of the functions under the guidelines. For many functions, the need for public review can be met through publication in the FEDERAL REGISTER. For certain responsibilities, comment may be solicited. Because procedures by which policies will be developed at the national and local levels are of key importance, notice is required for major policy initiatives.

Application to the Private Sector. Several commentators spoke on the application of the NIH guidelines to the private sector. Specifically, NIH was urged to provide voluntarily to private industry—

- Advice on interpretation of the guidelines,
- Registration of projects,
- Certification of host-vector systems,
- Advice on the operation of institutional biosafety committees, and
- Protection for patent and proprietary information.

In June 1976 representatives of private industry were invited to NIH to be briefed on the guidelines about to be released. Since their release, NIH has held several other meetings with representatives from the private sector. Commerce Department representatives on the interagency committee played a leading role in working with private industry on adoption of the safety standards of the NIH guidelines. All relevant industries have agreed to abide by those standards. However, many of the services provided to NIH grantees and contractors have not been extended to the private sector. In large part, efforts to do so have been held in abeyance because of possible Federal legislation.

After carefully considering the comments at the public hearing and in letters received, NIH will extend certain added services to the private sector in several of the areas suggested by the commentators. It is still important, despite proposed legislation, that the NIH provide for mechanisms to allow private-sector participation. Further, if legislation is enacted, the NIH guidelines will serve as the basis for regulation that will encompass private industry. Thus, a new section has been added to part IV that provides the opportunity for industry's participation in a voluntary fashion.

Office of the Director. As suggested by the commentators, the responsibilities of the Director have been grouped, for purposes of clarity, under specific heading "Office of the Director, NIH" in the PRG NIH. For many of the responsibilities cited including revision of the guidelines, certification of host vector systems, and authority for exemptions and exceptions appropriate notice and opportunity for public comment is specified. This opportunity for comment will provide structure for the exercise of discretion by the Director.

The PRG RAC clarified the relevant responsibilities of the NIH Director and RAC with regard to the certification of host-vector systems. Those concepts are adopted in the PRG—NIH.

Recombinant Advisory Committee. Many commentators have made suggestions concerning the structure, function, and scope of responsibility of the RAC. The emphasis in RAC membership has been on ensuring relevant scientific representation. It is essential that the committee have the technical expertise necessary to develop, modify, and interpret the guidelines in light of scientific evidence. Representative have been added from scientific disciplines, such as botany, to ensure a broad scientific overview. As a bridge between the implications for science and public policy, public members now serve on the committee, and additional public members may be added. Current public members are Dr. Emmette S. Redford, Ashbel Smith, professor of government and public affairs at the Lyndon B. Johnson School of Public Affairs, University of Texas, at Austin, and Dr. LeRoy Walters, director for the Center of Bioethics, Kennedy Institute, Georgetown University.

In order to insure fairness and sensitivity to the public commentators, procedures for nomination to the RAC will be in accord with the report by the NIH Grants Peer Review Study Committee. Thus, NIH will publish an announcement of upcoming vacancies periodically, with instructions on how to submit nominations. By this means, a wide spectrum of nominations will be considered to assure appropriate representation suited to the RAC's needs.

In brief, the operations of the RAC have been more clearly detailed in the PRG NIH. The procedures for the selection of members and the operations

of the committee are in the process of being formalized for the benefit of the scientific community and the public.

NIH Components. A new section in the PRG-NIH now describes all other functions of NIH, including the responsibilities of the Office of Recombinant DNA Activities (ORDA). It should be noted that the responsibility of the peer review groups (study sections) for an independent assessment of the recombinant DNA research protocols has been eliminated. This responsibility would be solely ORDA's in conjunction with the local institutional biosafety committee.

Several commentators urged new responsibilities for ORDA and additional personnel to fulfill them. Some recommended that the Office be responsible for inspecting and certifying laboratories at the P3 level. Currently NIH has the responsibility for certifying only P4 facilities. At present NIH operates, at the Frederick Cancer Research Center in Frederick, Md., and at NIH in Bethesda the only P4 facilities in this country. Responsibilities for certification falls to NIH because of the special nature of P4 facilities. P3 facilities, on the other hand, do not require special expertise at the national level, and there is no need for them to be nationally certified. As specified, the local institution has and should have responsibility for monitoring and certifying P1, P2, and P3 facilities.

Several commentators urged an increased flow of information to the public and scientific community alike. ORDA is playing a key role in disseminating information through the Recombinant DNA Technical Bulletin. This is a new publication that attempts to link investigators involved in recombinant DNA research, both in the United States and abroad, with the active advisory groups and organizations. In light of comments received, the bulletin will include in the future far more information for institutional biosafety committees and for the several advisory groups at the national and social levels.

In response to another suggestion, ORDA will be as available as possible to State and local governments for technical advice. Currently ORDA serves as a clearinghouse for information related to recombinant DNA activities internationally, nationally, and locally.

Registration and compliance

It has become clear over the 2 years of administration of the guidelines that a new section must be added on general requirements for registration with NIH. This should apply not only to NIH grantees and contractors, but also to the private sector on a voluntary basis. Further, in light of the review of DHEW policies on the patenting of recombinant DNA research inventions, a section on disclosure of information is also necessary. Finally, as suggested, a section on compliance with the guidelines is needed. Thus new sections C and D on registration (including disclosure of information) and compliance have been added to the roles and responsibilities section of the PRG-NIH. These provisions, recommended in many comments on the guidelines and at the December public hearing, are necessary in the absence of legislation.

Registration. A number of commentators asked that the guidelines specify the requirements for registration. Accordingly, a new subsection has been added delineating the elements. If other requirements need be added, notice will be given of any change. All projects subject to the guidelines must be registered with ORDA. Voluntary registration for the private sector is provided in the revision, in response to suggestions by private-sector representatives.

Disclosure. Many comments, as previously noted, were directed to the protection of proprietary information. A new subsection outlining the elements for protection of proprietary data is included in response to these suggestions.

One commentator urged that no patents be granted for recombinant DNA research.

Shortly after the release of the Guidelines in 1976, NIH received a letter requesting a review of DHEW policies relating to the patenting of recombinant DNA research inventions. The letter prompted NIH to review current patent regulations governing institutional patent agreements and to consider how recombinant DNA research inventions should be handled under those terms. On the basis of extensive Department and Interagency Committee review, it was agreed that, at least for the present, recombinant DNA research inventions developed under DHEW/NIH support should continue to be administered within current HEW patent agreements. Each agreement, however, would require assurance of compliance with the physical and biological containment standards set forth in the Guidelines as a condition for the granting of a license.

Policy on Noncompliance. A commentator urged that a system of fines be spelled out. Monetary fines, more appropriate for regulations under legislation, will not be specified or assessed under the Guidelines; NIH has no current authority to impose fines. It will, however, suspend, limit, or terminate a grant or contract for noncompliance. A commentator recommended that penalty procedures be specified. Should it be necessary to suspend, limit, or terminate a grant, appropriate HEW procedures will be followed.

In summary, Part IV of the Guidelines on Roles and Responsibilities has been substantially revised in response to suggestions from many commentators. The PRG-NIH now provides even more opportunity for advice from the local to the national level. The spirit of cooperation and effective overview will be enhanced by the PRG-NIH at the local level between the research community and the public and at the national level among Federal agencies, the scientific community, and the private sectors.

Proposed Action: Environmental Impact Assessment

The recommendations of the Recombinant Advisory Committee have been carefully weighed, along with other public and scientific comments received on the Roles and Responsibilities section. In general, the PRG-RAC proposals have been adopted in the PRG-NIH, with certain modifications based on issues raised by the Director's Advisory Committee and other commentators. The issues considered by the Director and a discussion of them follow.

The Draft EIS on the original Guidelines published in 1976 elicited a number of recommendations that greater detail be provided on NIH implementation of the Guidelines for NIH grantees and contractors. They also recommended extending the Guideline standards to all public and private sectors where such research is being conducted. More specifically, commentators expressed the following concerns.

- That the membership of institutional biohazards committees (IBCs) should include specialists in population dynamics, ecology, and other disciplines;
- That the Draft EIS did not emphasize relevant safety training for laboratory personnel;
- That the NIH Guidelines had a far too limited scope, not reaching research in the non-Federal sectors;
- That the termination of NIH funds for violation of the Guidelines may not be the best sanction;
- That the inspection, certification, and surveillance processes might not insure compliance;
- That more attention should be given to medical surveillance and epidemiologic measures in the event of possible infection of the laboratory worker or contamination of the environment; and
- That local and State authorities be involved in the review and containment processes at the local level.

These comments were specifically addressed in the Final EIS. It was

noted that the Guidelines established an administrative framework for assigning responsibility to insure safety in NIH-supported recombinant DNA research—a responsibility shared among principal investigators, their institutions, and NIH. The institutions were required to establish biohazards committees to carry out institutional responsibilities.

As discussed in the Final EIS, there were several factors contributing to the expectation that NIH grantees, contractors, and intramural scientists would comply with the Guidelines. They included the fact that noncompliance could result in the termination of funding; that investigators and their institutions share responsibilities for compliance; and that peer pressure on investigators for compliance would be accomplished through responsible institutional officers, local biohazards committees, and NIH review.

The Final EIS also discussed, in response to commentators, general Federal regulations of all such research to insure that work beyond the aegis of NIH would be done under the safety standards of the Guidelines. A Federal Interagency Committee, chartered by the Secretary of HEW with the approval of the President, was convened under the chairmanship of Dr. Donald S. Fredrickson, Director, NIH. In March 1977 that committee with representatives of all relevant research and regulatory agencies recommended to the Secretary of HEW that legislation be enacted to regulate all recombinant DNA research. HEW Secretary Califano had legislation developed in light of the committee's recommendations. An administration bill drafted by the Department was introduced in the Senate by Senator Edward M. Kennedy, Chairman of the Subcommittee on Health and Scientific Research of the Senate Committee on Human Resources, and in the House by Representatives Paul C. Rogers, Chairman of the Subcommittee on Health and the Environment of the Interstate and Foreign Commerce Committee. Congressional hearings were held, and respective committee bills were drafted, but not acted upon by Congress in its first session. New committee bills are pending congressional action.

The PRG-NIH, Parts I through III, reflect in large measure the safety of many of the experiments that would be exempt from the Guidelines or allowed at lower containment conditions. These changes are now proposed following an assessment that there would be no significant impact on the environment from the proposed revisions. The changes in Part IV largely reflect the concerns of the environmental commentators for greater emphasis on training in occupational and environmental safety and on explicit penalties for noncompliance. In addition, measures are provided that would allow private-sector engagement with the NIH, including registration of recombinant DNA activities.

To address occupational and environmental health and safety concerns, several changes are proposed in the implementation, review, and monitoring of recombinant DNA activities at the local and national levels, to insure appropriate safety practices and procedures that would minimize any significant environmental impact. These modifications primarily focus on a restructuring and amplification of Part IV, "Roles and Responsibilities," and an important and significantly expanded delegation of authority to local institutions.

These major changes from the 1976 Guidelines, which extend beyond the PRG-RAC, have resulted from a careful consideration of many comments received. The foremost concern during this process was to insure occupational and environmental safety, while at the same time refining the interdependent roles necessary to achieve this goal. Also, it was desirable to try to simplify and clarify administration of the Guidelines, as suggested by several commentators, thereby promoting a more successful application of their safety features. The revisions that have been proposed for Part IV represent a further step toward insuring the safe conduct of this research and minimizing the possibility of any untoward environmental effects.

In response to several requests for expanding the applicability of the guidelines, institutions receiving NIH funds for recombinant DNA projects and their "biosafety committees" (the proposed new designation for biohazards committees) are given responsibility for reviewing all recombinant DNA work conducted at the institution regardless of source of funds. This increase in the scope of review will better insure the safe conduct of recombinant DNA research.

Further, biosafety committees (IBCs) have been given broader responsibilities. The PRG-RAC proposed to allow the IBC's to approve single-step reductions in containment levels in experiments with purified DNA or characterized clones. This is retained in the PRG-NIH. In response to other comments, however, it is deemed necessary to specify IBC responsibilities for determining containment levels required by the guidelines. The PRG-NIH requires the IBC's to make independent evaluations of these containment levels. This should strengthen safety considerations locally.

It was also suggested that NIH delegate to biosafety committees the responsibility for approving or disapproving, on behalf of the institution, proposed recombinant DNA research, based on their independent assessment of the safety standards applied, and that IBC approval be sufficient for the research to proceed. NIH, through ORDA, would review all local committee actions to insure compliance with the guidelines, thereby maintaining national standards. This proposal has a number of advantages. It would simplify previous approval procedures and minimize delays due to NIH administration. Accountability for safe conduct of the research would reside at the local level, with appropriate Federal overview.

It is believed that incorporation of these recommendations for increased local authority would enhance implementation of the guidelines, since important responsibilities are clarified and more suitably located.

As mentioned earlier, another major change includes the restructuring and amplification of Part IV of the guidelines. Publication of the Director's decision on the 1976 guidelines, the draft EIS, and the PRG-RAC elicited numerous comments calling for more discussion and information on implementation, particularly for further clarification of responsibilities and roles at the local level. Accordingly, the contents of Part IV are presented in a format different from the current guidelines and the PRG-RAC, with the intent of more clearly alining the various duties.

At the request of several commentators, Appendix D, dropped from the PRG-RAC, has been revised and updated as "Laboratory Safety Monograph—A Supplement to the NIH Guidelines for Recombinant DNA Research." It provides a compendium of useful safety information, including instructions on emergency procedures, laboratory techniques for biohazard control, and decontamination and disposal methods. Expanded to include complementary information on roles and responsibilities, it provides directions for the operation of a biosafety committee, suggestions to increase the biological safety officer's efficiency, and more detail on the elements of a medical surveillance program. Criteria for certifying biological safety cabinets illustrate the level of detail. It is believed that the PRG-NIH, strengthened by these additions, provide a higher measure of protection for researchers, the public, and the environment.

Considerable attention has been focused on the institutional biosafety committees. Commentators have raised questions concerning the committees' role, membership, and operation. Consideration of various sugges-

tions has resulted in changes in the IBC membership requirements.

Noninstitutional representation is now proposed on biosafety committees, going beyond the PRG-RAC.

The role and responsibilities of the local biosafety committees have been restated and refined in response to numerous comments. They are also strengthened by the required membership of the biosafety officer. The PRG-RAC proposed the designation of a biological safety officer for each institution where P3- and P4-level recombinant DNA research was conducted, reflecting the practice of a growing number of American institutions and the recommendations of the British "Williams Committee Report." The requirement is intended to insure that a clearly designated individual will have the primary administrative responsibility for the implementation of institutional policies and biosafety committee decisions.

A related issue which drew several comments and recommendations concerned medical surveillance, or health monitoring. Responsibility for determining medical surveillance procedures for research personnel was transferred from the principal investigator to the institution, because the RAC felt that institutions would have access to a broader range of expertise.

Specific information on medical conditions was moved to the laboratory safety monograph, which includes additinal recommendations on health monitoring and program content. General language describing the elements of a medical surveillance program was retained in Part IV as part of the institution's responsibility.

The addition of information and the further emphasis on health monitoring should enhance occupational health and safety at the local level. It should be noted that a collaborative effort is underway between NIH and the Center for Disease Control to establish a mechanism for providing emergency teams of experts to respond to major laboratory accidents.

Probably no other issue generated as much comment as that of training. According to one commentator, training "probably represents the key to safety." He goes on to say that any break in technique could destroy physical containment procedures. Indeed, training in good microbiological practices could be considered the first line of defense, as it serves to protect the worker as well as prevent contamination of the environment inside and outside the laboratory. The PRG-RAC proposed the addition of a requirement for the training of research personnel in the use of good microbiological technique. In the PRG-NIH, the principal investigator must be trained, and must insure the training of laboratory personnel, before undertaking recombinant DNA experiments. The laboratory safety monograph includes a section on "Laboratory Techniques for Biohazard Control" which describes good practices regarding pipetting, centrifuging, and the use of syringes and needles. Altogether, training requirement s have been further emphasized and strengthened in the PRG-NIH.

Several commentators wanted training standards to be set in the Guidelines or by NIH, the institution, or the biosafety committees. Others opposed setting standards. Still others recommended mandatory, formal evaluation and certification of investigators to test for competency in microbiological techniques. NIH is currently funding a Working Panel of the American Society of Microbiology (ASM) which is considering standards of training in microbiological techniques for recombinant DNA research. Plans call for the dissemination of a report for the use of institutions, IBC's, and principal investigators, once NIH receives the recommendation of the ASM Panel.

NIH considers it premature to require national certification, as suggested by some. There are so few models for formal training standards in biomedical research that NIH has decided to proceed cautiously, while continuing to promote safety training. As yet, there is no consensus, no one best way to accomplish this training, and therefore the desired results may best be achieved by allowing institutions, biosafety committees, and investigators some flexibility. Meanwhile, NIH plans to develop courses to be based on the NIH/ASM standards and to offer them widely. The Laboratory Safety Monograph also contains a summary of safety training aids, materials, and courses offered by the NIH and others.

From the discussion of these issues, it should be evident that measures have been undertaken in the revision of Part IV of the Guidelines to emphasize occupational health and safety. The principal measures would be the strengthening of training requirements and activities, the designation of someone in the institution as a member of the biosafety committee to handle biological safety questions generally, the delegation of more authority to the institution, and better definition of responsibilities at the local level. The impact of these actions will be the promotion of safer conduct of this research, thus affording a greater measure of protection to the environment.

In summary, a key focus of public comment was on "procedural due process" to insure public participation in the development of NIH recombinant DNA policies. In response to those suggestions in several instances, opportunity for public comment is afforded through activities of the Director, NIH, and the RAC. In addition, public representation on the RAC will be considered further, with a nomination process to insure a wide range of individuals for selection. New general compliance and registration sections have been included in the PRG-NIH that do not appear in the current Guidelines. These sections are directly responsive to stated concerns that the importance of these safety standards and procedures be emphasized.

NIH will continue to work closely with other relevant research and regulatory agencies, particularly the Environmental Protection Agency and the Occupational Safety and Health Administration. Both of these agencies are represented on the Federal Interagency Committee on Recombinant DNA Research.

NIH has created over the past 2 years several internal committees that are critically examining areas where work with potential biohazards is involved in the laboratory.

Finally, a key concern of all commentators was the need for programs in occupational and environmental safety which would include health surveillance for laboratory personnel and the community. Recombinant DNA research policies have stimulated a broad NIH commitment and interest in laboratory safety. NIH has a mandate for national leadership in laboratory safety programs, and the proposed revisions reflect full acceptance of that responsibility.

APPENDIX A-1

Table: Comparison of Containment Levels

The following table compares the containment levels for all permissible types of recombinant DNA experiments. It designates the levels under the Guidelines now in effect (since June 1976), under those proposed by the RAC (September 1977), and under the present NIH-proposed revisions.

A dash (—) indicates that the category is not classified in the edition of the Guidelines under which the dash appears.

It should be stressed that the table is not definitive, since the containment levels of the Guidelines have been redefined and other requirements modified.

Appendix A

COMPARISON OF THE CONTAINMENT LEVELS OF THE 1976 GUIDELINES AND THE PROPOSED REVISED GUIDELINES

INSERTED DNA	CONTAINMENT LEVELS					
	JUNE 1976 GUIDELINES		RAC PROPOSED REVISED GUIDELINES		NIH DIRECTOR'S REVISED GUIDELINES	
	PHYSICAL	BIOLOGICAL	PHYSICAL	BIOLOGICAL	PHYSICAL	BIOLOGICAL
Shotgun Experiments						
1. Primate	P4 or P3	EK2 EK3	P4 or P3	EK1 EK3	P2 —	EK2[1] —
2. Primate DNA from uninfected cells	—	—	P3	EK2	—	—
3. Embryonic primate	P3	EK2	—	—	—	—
4. Mammals (other than primates)	P3	EK2	P2	EK2	P2	EK2
5. Birds	P3	EK2	P2	EK2	P2	EK2
6. Cold blooded vertebrates	P2	EK2	P3 or P2	EK1 EK2	P2 or P1	EK1 EK2
7. embryonic and germ line	P2	EK1	P2 or P1	EK1 EK2	—	—
8. producing potent toxins	P3	EK2	—	—	—	—
9. producing potent polypetide toxins	—	—	P3	EK2	P3	EK2
Lower eukaryotes						
10. producing toxins, or are pathogens	P3	EK2	—	—	—	—
Shotgun Experiments (Cont.)						
11. producing potent polypeptide toxins, or are pathogens	—	—	P3	EK2	P3	EK2
12. producing non-polypeptide toxins	—	—	P3 or P2	EK1 EK2	P3 or P2	EK1 EK2
13. producing toxins affecting invertebrates or plants.	—	—	P3 or P2	EK1 EK2	P3 or P2	EK1 EK2
14. remainder of species	P2	EK1	P2 or P1	EK1 EK2	P2 or P1	EK1[2] EK2
15. Plants	P2	EK1	P2 or P1	EK1 EK2	P2 or P1	EK1 EK2
16. carrying pathogens or making dangerous products	P3	EK2	—	—	—	—
17. carrying pathogens or making polypeptide toxins	—	—	P3	EK2	—	—
18. making potent polypeptide toxins	—	—	—	—	P3	EK2
19. making non-polypeptide toxins	—	—	P3 or P2	EK1 EK2	P3 or P2	EK1 EK2

COMPARISON OF THE CONTAINMENT LEVELS OF THE 1976 GUIDELINES AND THE PROPOSED REVISED GUIDELINES

INSERTED DNA	CONTAINMENT LEVELS					
	JUNE 1976 GUIDELINES		RAC PROPOSED REVISED GUIDELINES		NIH DIRECTOR'S REVISED GUIDELINES	
	PHYSICAL	BIOLOGICAL	PHYSICAL	BIOLOGICAL	PHYSICAL	BIOLOGICAL
Prokaryotes exchanging genetic information with E. coli						
20. Class 1 CDC agents	P1	EK1	—	—	P1	EK1[3]
21. Class 2 CDC agents	P2 or P2	EK1 EK2	—	—	P2	EK1[3]
22. Prokaryotes not exchanging genetic information with E. coli	P3 or P2	EK1 EK2	See Prokaryotic DNA recombinants (Categories 24-26)		P2 or P1	EK1 EK2
23. If pathogenic species	P3	EK2			P3	EK2
Prokaryotic DNA recombinants						
24. source of DNA is extensively characterized	—	—	P2 or P1	EK1 EK2	—	—
25. source of DNA is not extensively characterized	—	—	P2 or P3	EK2 EK1	—	—
26. source of DNA is pathogenic species	—	—	P3	EK2	—	—
Characterized clones						
27. Species that exchange information with E. coli	P1	EK1	—	—	See Characterized clones (Category 50)	
28. Species that do not exchange with E. coli	P2	EK1	See Characterized clones (Category 50)		See Characterized clones (Category 50)	
29. Purified DNA other than Plasmids, Bacteriophages, and Other Viruses:	One step reduction in physical or biological containment from that used in corresponding shotgun experiments		Same		Same, except for primate DNA (See Footnote #1)	
Plasmids, Bacteriophages and Other Viruses						
30. Animal viruses	P4 or P3	EK2 EK3	See Categories 32-41		*	
31. When clones shown to be free of harmful regions	P3	EK2	See Categories 32-41		*	
Viruses of Warm-Blooded Vertebrates						
32. DNA viruses + transcripts of retrovirus genomes	—	—	P4 or P3	EK1 EK3	*	

COMPARISON OF THE CONTAINMENT LEVELS OF THE 1976 GUIDELINES AND THE PROPOSED REVISED GUIDELINES

	INSERTED DNA	CONTAINMENT LEVELS					
		JUNE 1976 GUIDELINES		RAC PROPOSED REVISED GUIDELINES		NIH DIRECTOR'S REVISED GUIDELINES	
		PHYSICAL	BIOLOGICAL	PHYSICAL	BIOLOGICAL	PHYSICAL	BIOLOGICAL
33.	for purified subgenomic segments or less than complete genome	—	—	P3	EK2		*
	DNA transcripts of other RNA viruses						
34.	non-segmented genome	—	—	See Categories 32-41			*
35.	segmented genome	—	—	P3	EK2		*
	Viruses of Cold-Blooded Vertebrates						
36.	DNA viruses + transcripts of retrovirus genomes	—	—	P4 or P3	EK1 EK2		*
37.	for purified subgenomic segments or less than complete genome	—	—	P2	EK2		*
	DNA transcripts of other RNA viruses						
38.	non-segmented genome	—	—	See Categories 36-37			*
39.	segmented genome	—	—	P2	EK2		*
	Viruses of Invertebrates and Protozoa						
40.	Baculoviruses, EPA registered	—	—	P1 or P2	EK2 EK1		*
41.	Other viruses	—	—	Same as for viruses of cold-blooded vertebrates			*
42.	Plant viruses	P3 or P2	EK1 EK2	P3 or P2	EK1 EK2		*
43.	Primate mitochondria	P3 or P2	EK1 EK2	P3 or P2	EK1 EK2	—	—
44.	Other eukaryotic mitochondrial or chloroplast DNA	P2	EK1	P2	EK1	—	—
45.	Eukaryotic mitochondrial or chloroplast DNA	—	—	—	—	P2	EK1
46.	Prokaryotic plasmid or phage which exchange with E. coli	Same as shotgun conditions for host		—	—	Same as shotgun conditions for host	
47.	when known not to contain harmful genes, or purified segments	P1	EK1	—	—	(See Footnote #3)	

COMPARISON OF THE CONTAINMENT LEVELS OF THE 1976 GUIDELINES AND THE PROPOSED REVISED GUIDELINES

	INSERTED DNA	CONTAINMENT LEVELS					
		JUNE 1976 GUIDELINES		RAC PROPOSED REVISED GUIDELINES		NIH DIRECTOR'S REVISED GUIDELINES	
		PHYSICAL	BIOLOGICAL	PHYSICAL	BIOLOGICAL	PHYSICAL	BIOLOGICAL
48.	Prokaryotic plasmid and phage which do not exchange information with E. coli	Same as shotgun conditions for host		Same as shotgun conditions for host		Same as shotgun conditions for host	
49.	minimum containment	P3 or P2	EK1 EK2	—	—	—	—
50.	Characterized Clones	See Characterized clones (Category 27-28)		IBC may approve single-step reduction in physical or biological containment		IBC may approve single-step reduction in physical or biological containment, except for primate DNA	
				Reduction of more than one step requires NIH approval		Reduction of more than one step, or reduction of primate DNA requires NIH approval.	
	Animal Host-Vector Systems						
	Defective polyoma virus						
51.	Containing DNA of non-pathogenic organism	P3	cultured cells	See Categories 58-60		**	
52.	Containing class 2 animal virus sequences	P4	cultured cells	See Categories 58-60		**	
53.	when clone shown to contain only harmless regions	P3	cultured cells	See Categories 58-60		**	
	Defective SV40 virus						
54.	DNA from any non-pathogenic organism or Class 1 virus	P4	established cell lines	See Categories 61-65		**	
55.	if inserted segment is purified prokaryotic DNA or previously cloned eukaryotic DNA whose function is known	P3	established cell lines	See Categories 61-65		**	
56.	if defective SV40 lacks late region, there is no helper virus, and no infectious virus is being produced	P3	established cell lines	See Categories 61-65		**	
57.	if non-permissive cells transformed	P3	established non-permissive cell lines	See Categories 61-65		**	
	Defective or intact polyoma virus						
58.	Containing DNA of Class 1 or Class 2 bacteria, except for species producing potent polypeptide toxins	—	—	P3	cultured cells	**	

COMPARISON OF THE CONTAINMENT LEVELS OF THE 1976 GUIDELINES AND THE PROPOSED REVISED GUIDELINES

	INSERTED DNA	CONTAINMENT LEVELS					
		JUNE 1976 GUIDELINES		RAC PROPOSED REVISED GUIDELINES		NIH DIRECTOR'S REVISED GUIDELINES	
		PHYSICAL	BIOLOGICAL	PHYSICAL	BIOLOGICAL	PHYSICAL	BIOLOGICAL
59.	Containing DNA of eukaryote not producing potent polypeptide toxin	—	—	P3	cultured cells	**	
	Unconditionally defective polyoma virus						
60.	Subgenomic segments of Class 1 or Class 2 viruses which do not replicate in mouse cells	—	—	P3	cultured cells	**	
	SV40 and Adenovirus Types 2 and 5						
	Unconditionally defective by deletion in essential gene region						
61.	if inserted DNA is from Class 1 or 2 bacteria (except for species producing potent polypeptide toxins) or from eukaryote not producing potent polypeptide toxin	—	—	P4	cultured cells	**	
62.	if inserted DNA is purified prokaryotic segment, or identified segment of eukaryotic DNA previously cloned in prokaryotic host-vector system	—	—	P3	cultured cells	**	
63.	Unconditionally defective by deletion in capsid genes						
	containing DNA from Class 1 agents	—	—	P4	established cell line	**	
64.	if vector is known to be free of complete viral genome	—	—	P3	established cell line	**	
65.	SV40 or Ad. 2 and 5 DNA plus subgenomic sequences from eukaryotic organisms or Class 1 or 2 agents; recombinant molecule defective and no virus being produced	—	—	P3	non-permissive cells	**	

APPENDIX A

FOOTNOTES

1. Lowering of containment below these levels (i.e., for purified DNA or characterized clones) cannot be made solely by an institutional biosafety committee, but requires NIH approval.

2. Plant pathogenic or synbiotic fungi that do not produce potent toxins are specifically included among the remainder of species in this class of lower eukaryotes.

3. It is expected that many of the prokaryotes that exchange genetic information with *E. coli* by known physiological processes will be exempted from these Guidelines by appearing on the "list of exchangers" (See Guidelines, Section I-E-4.)

*See table III of Guidelines, Section III-A-2-a-(2)-(e).

**See table IV of Guidelines, Section III-C-3.

APPENDIX B-1

Table: Classification of NIH-Funded Experiments as of December 1976

The following table lists all P2 and P3 recombinant DNA research projects supported by NIH as of December 1977. The P1 projects are roughly a 50-percent sample. The table designates the containment labels under the Guidelines now in effect (since June 1976), under those proposed by the RAC (September 1977), and under the present NIH-proposed revisions.

Appendix B--2

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P2 + EK1	B. subtilis	K-12, C600*	pBR313	?Exempt	P1 + EK1
P2 + EK1 or EK2	S. typhimurium	K-12	K-12	?Exempt	Exempt
P2 + EK1	Sea Urchin	K-12	pSC101, pSP2	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Slime Mold	K-12	λ, pSC101 pMB9	P2 +EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Maize Chloroplast	K-12, HB101*	various plasmids	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Neurospora	K-12	pMB9, pML21	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Embryonic Xenopus	K-12	pMB9, pSC101	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
	Characterized Xenopus	"	"	P2 + EK1	P1 + EK1
P2 + EK1	B. subtilis	K-12	pMB9, pSC101	?Exempt	P1 + EK1
P2 + EK2	Embryonic Xenopus	χ1776	pMB9	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
	Characterized Xenopus	"	"	P2 + EK1	P1 + EK1

Appendix B--3

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P2 + EK2	S. typhimurium	K-12	λ, Col E1	?Exempt	Exempt
P2 + EK2	Characterized Xenopus	K-12	λ	P2 + EK1	P1 + EK1
P2 + EK1	Mouse Mitochondria	C600*	pSC101, pMB9	P2 + EK1	P2 + EK1
P2 + EK1	Drosophila	K-12	various plasmids	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Paramecium	K-12, HB101*	pMB9, λ	P2 + EK1	P2 + EK1
P2 + EK1	Sea Urchin	K-12	λ	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK2 or P3 + EK1	Actinomycetes	K-12	λ, Col E1	P2 + EK2 or P3 + EK1	P3 + EK2
P2 + EK2 or P3 + EK1	Streptomyces	K-12, χ1776	pSC101	P2 + EK2 or P3 + EK1	P2 + EK1 or P1 + EK2

Appendix B--4

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P2 + EK1	Drosophila	K-12	pSC101, Col E1	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Sea Urchin	K-12	pMB9, pBR322, pSP2124, pBGP120	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK2	Triturus cristatus	C600*	pMB9, pSC101, pCRi	P2 + EK2 or P3 + EK1	P2 + EK2 or P3 + EK1
P2 + EK1	Notophthalmus viridescens	"		P2 + EK1 (RAC decision)	P2 + EK1
P2 + EK1	Silkworth	HB101*	pML21	P2 + EK1 (RAC decision)	P2 + EK1
P2 + EK1	B. subtilis	C600*	pBR313	?Exempt	P1 + EK1
P2 + EK1	Drosophila	K-12	Col E1	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
	Embryonic Xenopus	K-12	pSC101, Col E1	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2

Appendix B--5

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P2 + EK1	Neurospora	K-12	pMB9, pBR322, Col E1	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Drosophila	C600*	pMB9	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Drosophila	K-12	pMB9, pSC101, Col E1	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	B. subtilis	C600*	pMB9, pSC101, pBR322	?Exempt	P1 + EK1
P2 + EK1	Drosophila	K-12	pMB9	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Drosophila	K-12	λ	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Yeast	K-12, C600*	Col E1	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2

Appendix B--6

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P2 + EK1	Drosophila	HB101*	pMB9	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Yeast	K-12	pMB9, pBR313	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EL2
P2 + EK1	Drosophila	K-12, HB101*, 5P8	pMB9, pBR313	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Neurospora	K-12	pMB9, Col E1	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Neurospora	C600*	λ, Col E1	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Drosophila	K-12	pBR322, RSF2124	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Caulobacter crescentus	K-12	λ, RSF2124	?Exempt	~~Exempt~~ P1 + EK1

Appendix B--7

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P2 + EK1	Yeast	C600*, RR1*	λ, Col E1	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Tetrahymena	HB101*, 5P8*	pBR313, RSF2124, pMB9	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
	Yeast				
	Drosophila	"	"	"	"
	Embryonic Ambystoma (Amphibian)	"	"	"	"
	Characterized Xenopus	"	"	P2 + EK1	P1 + EK1
	Characterized rabbit (pBG1)	"	"	P2 + EK1 (RAC decision)	P2 + EK1
	Triturus	"	"	"	"
P2 + EK1	Slime Mold	K-12	λ, pMB9, pSC101, Col E1	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Yeast	K-12	λ, pMB9, pBR322, Col E1	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	S. typhimurium	K-12	λ, pMB9, pBR322, Col E1	?Exempt	Exempt

Appendix B--8

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P2 + EK1	Mouse Mitochondria	C600*	pSC101, Col E1	P2 + EK1	P2 + EK1
P2 + EK1	Slime Mold	C600*, HB101*, SF8*	λ, pMB9, pBR313, pBR322	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Drosophila	K-12	Col E1	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Yeast	K-12	pMB9	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Drosophila	K-12	pBR313	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Soybean	HB101*, RR1*	pBR322, pBR313, pSC101, pAC184	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Yeast	K-12	RSF2124, pBR322	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2

Appendix B--9

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P2 + EK1	Characterized Xenopus	C600*, χ1776	pSC101, Col E1	P2 + EK1	P1 + EK1
	Sea Urchin			P2 + EK1 or P2 + EK2	P2 + EK1 or P1 + EK2
	Mouse Mitochondrial			P2 + EK1	P2 + EK1
	Drosophila			P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
	Characterized staphylococcus			P2 + EK1	P2 + EK1
P2 + EK1	Yeast	K-12	pCR1	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Yeast	DC51*	pMB9	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Sea Urchin	K-12	pSC105	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Leishmania (protozoan)	K-12	pSC101, Col E1	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2

Appendix B--10

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P2 + EK1	Sea Urchin Psammechinus milaris	K-12	λ, pSC101, pSP2, pSP17	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P1 + EK1	E. coli (enteropathogenic)	K-12, C600*, HB101*	pSC101, RSF 2124, pBR313, pBR322, pBR333	?Exempt	Exempt
P2 + EK1	B. subtilis	K-12	Col E1	?Exempt	P1 + EK1
P2 + EK1	Bean	K-12	λ, Col E1	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P1 + EK1	E. coli (enteropathogenic)	K-12	Col E1	?Exempt	Exempt
P2 + EK1	B. subtilis	K-12	K-12 plasmids	?Exempt	P1 + EK1
P2 + EK1	S. typhimurium	K-12	λ, pMB9, pSC101	?Exempt	Exempt
P2 + EK1	Yeast	C600*	λ, pSC101, pMB9, Col E1	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2

Appendix B--11

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P2 + EK1	Drosophila Sea Urchin	K-12	pSC101, Col E1	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
	Characterized Xenopus	K-12	pSC101, Col E1	P2 + EK1	P1 + EK1
P2 + EK1	Drosophila Sea Urchin	K-12	pSC101, pMB9, pBR313	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Frog Mitochondria	HB101*	pMB9	P2 + EK1	P2 + EK1
P2 + EK1	Embryonic Frog	HB101*	pMB9	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Amoeba	HB101*	pBR313	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Yeast	K-12	pBR313	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2

Appendix B--12

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P2 + EK1	Yeast Drosophila	SF8*, HB101*	pMB9, pBR313	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	B. subtilis	SF8*	pMB9	?Exempt	P1 + EK1
	B. megaterium			?Exempt	P1 + EK1
	Aerobacter cloacae			?Exempt	P1 + EK1
	Aeromonas formicas			?Exempt	P1 + EK1
	Strep. lactis			?Exempt	P1 + EK1
	Yeast			P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Yeast	HB101*	p2124	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Yeast	HB101*	pMB9, pBR313	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2

Appendix B--13

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P2 + EK1	Embryonic Toad	HB101*	pMB9	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Corn	K-12	λ	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Sea Urchin Yeast	K-12	λ, pSC101	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Drosophila	K-12, HB101*	pSC101, 105, pMB9, pML2, pCR1, Col E1	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Drosophila	K-12 C600*	pMB8, pMB9, pBR322	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Yeast	HB101*	pMB9	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Neurospora	K-12	pBR313, pBR322	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2

Appendix B--14

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P2 + EK1	Corn	K-12	pBR322	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Drosophila	K-12	pMB9, pBR322, pSC101, Col E1	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Yeast	K-12	pMB9	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Sea Urchin	C600*	pSC101, Col E1, pGM16, pML21	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Drosophila Sea Urchin	K-12	pSC101, pDGP120	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Yeast	K-12	λ, pMB9, pBR313	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2

Appendix B--15

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P2 + EK1	Protozoan Yeast Drosophila Sea Urchin	K-12	pSC101, Col E1	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	S. typhimurium	RR1*	pBR313, 322	?Exempt	Exempt
P2 + EK1	Rabbit	K-12	pGB1	P2 + EK1 (RAC decision)	P2 + EK1
P2 + EK1	Neurospora	K-12	pMB9, Col E1, pSC101	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Embryonic Salmon	K-12	pMB9, Col E1, pSC101	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Sea Urchin	C600*	pSC101, Col E1, pGM16, pML21	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Drosophila	HB101*, χ1776	pMB9, pRI	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	B. subtilis	χ1776	pSC101	?Exempt	P1 + EK1

Appendix B--16

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P2 + EK1	Yeast	K-12	pMB9, pML21, λ, pRB313	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	S. typhimurium	K-12	pMB9, pML21, λ, pRB313	?Exempt	Exempt
P2 + EK1 (NIH approved)	B. subtilis	B. subtilis, RC138	pIM13	?Exempt	Exempt
P2 + EK1	Caulobacter crescentus	K-12	pMB9, pSC101	?Exempt	~~Exempt~~ P1 + EK1
P2 + EK1	B. subtilis	C600*	pMB9	?Exempt	~~Exempt~~ P1 + EK1
P2 + EK1	Drosophila	HB101*	pMB9, pBR313	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Protozoan	HB101*	pBR322	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Drosophila	C600*, x1776,	pSC101, pSC105, pMB9, pML2, pCRi, Col E1	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2

Appendix B--17

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P2 + EK1	Corn	K-12	λ	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	B. subtilis	K-12	pBR313, pMB9, pIM2	?Exempt	P1 + EK1
P2 + EK1	Drosophila	K-12, x1776	pSC101, pMB9, Col E1	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Drosophila	K-12	pMB9, pSC101, pBR322	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Characterized Toad	K-12	pSC101, 105, NSF2124	P2 + EK1	P1 + EK1
P2 + EK1	Protozoan	HB101*	pBR313, pBR322, pMB9	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Neurospora	K-12	pBR313, pBR322, pMB9, Col E1	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2

Appendix B--18

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P2 + EK1	Yeast	K-12	pMB9, pSC101, Col E1	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Drosophila	C600*	pMB9	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Klebsiella pneumoniae	K-12	pSC101, Col E1	?Exempt	Exempt
P2 + EK1	Yeast	K-12, χ1776	pMB9	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	S. typhimurium	K-12, C600*	Col E1	?Exempt	Exempt
P2 + EK2	Toad	K-12	pMB9, pSC101	P2 + EK2 or P3 + EK1	P2 + EK2 or P3 + EK1
P2 + EK1	Sea Urchin	K-12	λ	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Yeast	C600*	λ, pSC101, pMB9, Col E1	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2

Appendix B--19

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P2 + EK1	Drosophila	C600*	pSC101	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Gnat	K-12	pBR313	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Protozoan	K-12	pMB9, pBR322	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Yeast	K-12	Col E1	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	S. typhimurium	K-12	pBR322, pBR317	?Exempt	Exempt
P2 + EK1	Yeast	K-12	pMB9	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2

Appendix B--20

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P2 + EK1	Sea Urchin	K-12	pSP2, pSP17	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Moure Mitochondria	K-12	pSC101	P2 + EK1	P2 + EK1
P2 + EK1	Embryonic Xenopus	C600*	pCR1, pSC101, Charon 4A	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Yeast	E. coli	λ, pBR322	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Sea Urchin	K-12	2124	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1	Chloroplast	K-12	pSC101, Col E1	P2 + EK1	P2 + EK1
P2 + EK1	Chloroplast	K-12	2124	P2 + EK1	P2 + EK1

Appendix B--21

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P2 + EK1	Yeast	C600* HB101*	λ, pMB9	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1 (RAC approved)	Chicken	K-12	λ	P1 + EK1 (RAC approved)	P1 + EK1 (RAC approved)
P2 + EK1	Yeast	K-12, HB101*, SF8*	λ	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1 (RAC approved)	Rabbit Globin	χ1776, K-12	pMB9	P2 + EK2	P2 + EK2
P2 + EK1 (RAC approved)	Chicken	HB101*	pHB1001, pHB1003	P2 + EK1 (RAC approved)	P2 + EK1 (RAC approved)
P2 + EK1	Yeast	K-12	Col E1	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2
P2 + EK1 (RAC approved)	Human Globin	HB101*, C600*	JW102	P2 + EK1 (RAC approved)	P2 + EK1 (RAC approved)
P2 + EK1	Yeast	K-12	Col E1	P2 + EK1 or P1 + EK2	P2 + EK1 or P1 + EK2

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P3 + EK2	Mouse	χ1776	pCR1	P2 + EK2	P2 + EK2
P3 + EK2	Mouse	χ1776	pBR322	P2 + EK2	P2 + EK2
P3 + EK2	Chicken	χ1776	pBR322	P2 + EK2	P2 + EK2
P3 + EK2	Hamster	χ1776	pCR1, pSC101	P2 + EK2	P2 + EK2
P3	Rabbit Globin DNA	Cultured Monkey Cells	Defective SV40	P3	P3
P3		Non-Permissive Mouse Cells	Defective SV40	P3	P2

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P3 + EK2	Mouse	χ1776	pCR1	P2 + EK2	P2 + EK2
P3 + EK2	Rat	χ1776	pMB9, pBR322, pBR313	P2 + EK2	P2 + EK2
P3 + EK2	Human Embryo	"	"	P2 + EK2	P2 + EK2
P3 + EK2	Chicken	"	"	P2 + EK2	P2 + EK2
P3 + EK2	Human Embryo	χ1776 DH50/supF	pMB9, λgtWES.λB	P3 + EK2	P2 + EK2
P3 + EK2	Chicken	χ1776	pMB9	P2 + EK2	P2 + EK2
P3 + EK2	Mouse	χ1776	pCR1, pSC101	P2 + EK2	P2 + EK2
P3	Yeast Drosophila Mouse	Cultured Mouse and Hamster Cells	Defective SV40 and Polyoma	P3	P2

Appendix B--24

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P3 + EK2	Chicken	x 1776	pMB9, pCRl, Col El	P2 + EK2	P2 + EK2
P3 + EK2	Bird	x 1776	pSC101	P2 + EK2	P2 + EK2
P3 + EK2	Mouse Sarcoma cDNA	x 1776	pSC101, pMB9, pBR313, pBR322	P2 + EK2	P2 + EK2
P3 + EK2	Hamster	x 1776	pSC101	P2 + EK2	P2 + EK2
P3 + EK2	Chicken	DP50/supF	Charon 4A Charon 16A	P2 + EK2	P2 + EK2
P3 + EK2	Bird	x 1776	pCRl	P2 + EK2	P2 + EK2
P3 + EK1 P2 + EK1	Rabbit Globin pHGl	K-12	pMB9, pBR322	P2 + EK1 (RAC decision)	P2 + EK1
P3 + EK2	Mouse Embryo	DP50	Charon 16A	P2 + EK2	P2 + EK1

Appendix B--25

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P3 + EK1	Hela Mitochondria	C600*	pSF2124	P2 + EK2 or P3 + EK1	P2 + EK1
P3 + EK2	Mouse cDNA	x1776 or DP50	pCRl or Charon 16A	P2 + EK2	P2 + EK2
P3 + EK2	Chicken Rat	x1776	pSC101, pCRl	P2 + EK2	P2 + EK2
P3 + EK2	Mouse	x1776	pMB9, pBR322, pBR313	P2 + EK2	P2 + EK2
P3	E. coli	Mammalian Cells	Defective SV40	P3	P2
P3	E. coli	Cultured Monkey Cells	Defective SV40	P3	P2
P3 + EK2	Mouse	x1776	pCRl	P2 + EK2	P2 + EK2

Appendix B--26

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P3 + EK2	Goat	EK2	EK2	P2 + EK2	P2 + EK2
P3 + EK2	Rat	χ 1776	pMB9, pBR322	P2 + EK2	P2 + EK2
P3 + EK2	Rabbit	HB101*	pMB9	P2 + EK2	P2 + EK2
P3 + EK2	Rat	χ 1776 or DP50	pCR1 or Charon 16A	P2 + EK2	P2 + EK2
P3 + EK2	Mouse	EK2	EK2	P2 + EK2	P2 + EK2

Appendix B--27

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P3 + EK2	Rat	χ 1776	pBR322	P2 + EK2	P2 + EK2
P3 + EK2	Mouse Cow	χ 1776	pSC101, pCR1	P2 + EK2	P2 + EK2
P3 + EK2	Human Embryo	χ 1776	pSC101, pCR1	P3 + EK2	P2 + EK2
P3 + EK2	Rat, Dog, Mouse, Chicken, Pig, Human Embryo, Dog, Pig, Mouse, Rat, Chicken, Human Fetal	χ 1776	pBR322, pMB9	P3 + EK2	P2 + EK2

Appendix B--28

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P3 + EK2	Mouse	K 12	EK2	P2 + EK2	P2 + EK2
P3 + EK2	Chicken	EK2	EK2	P2 + EK2	P2 + EK2
P3 + EK2	Rabbit, Mouse, Sheep	χ1776	μM9	P2 + EK2	P2 + EK2
P3	λ	Trans-foumed Rat Cells	Defective SV40	P3	P2
P3 + EK1 (RAC approved)	Chicken	DF50/supF χ1776	Charon 4A, λgtWES.λB μBR322	P2 + EK2 or P3 + EK1 (RAC approved)	P2 + EK2 or P3 + EK1
P3 + EK2	Chicken, Mouse, Rat	χ1776	μBR322, μBR313	P2 + EK2	P2 + EK2
P3 + EK2	Human Sperm and Placenta	DF50/supF, χ1776	λgtWES.λB, μM9, μCR1, μBR322	P3 + EK2	P2 + EK2
P3	Rabbit, Mouse	Permissive Mouse Cells	Defective Polyoma Virus	P3	P2

Appendix B--29

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P3	Mouse, Rabbit	Mouse cells	Defective SV40	P3	P2
P3 + EK2	Chicken	DF50/supF	λgtWES.λB	P2 + EK2	P2 + EK2
		χ1776	μM9, μCR1	P2 + EK2	P2 + EK2
P3 + EK2	Purified Monkey	χ1776	μBR313, μBR322	P2 + EK2 or P3 + EK1	P1 + EK2 or P2 + EK1
P3 + EK2	Dog, Rat, Murine Cells	χ1776, DF50/supF	EK2	P2 + EK2	P2 + EK2

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P1 + EK1	K-12	K-12, r'600*	λ, P1	Exempt	Exempt
P1 + EK1	E. coli, λ, P1	K-12	λ	Exempt	Exempt
P1 + EK1	E. coli	K-12	Col E1, λ	Exempt	Exempt
P1 + EK1	E. coli	K-12	Col E1	Exempt	Exempt
P1 + EK1	E. coli	K-12	φ80, λ, Col E1	Exempt	Exempt
P1 + EK1	E. coli	K-12	pBC201	Exempt	Exempt
P1 + EK1	E. coli, φ80	K'411*	pSC101	Exempt	Exempt
P1 + EK1	E. coli, λ	K-12	2124, Col E1	Exempt	Exempt
P1 + EK1	λ	K-12	pMB9, pBR322	Exempt	Exempt
P1 + EK1	K-12, λ	K-12, RRI*	λ	Exempt	Exempt
P1 + EK1	λ, Col E1	K-12	λ, pBC201	Exempt	Exempt
P1 + EK1	K-12	K-12	pSC101, pBR322	Exempt	Exempt

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P1 + EK1	E. coli	K-12	Col E1	Exempt	Exempt
P1 + EK1	E. coli	K-12	λ	Exempt	Exempt
P1 + EK1	E. coli	K-12	λ	Exempt	Exempt
P1 + EK1	E. coli	K-12	λ, Col E1, pSC101, P1	Exempt	Exempt
P1 + EK1	λ	K-12	Col E1	Exempt	Exempt
P1 + EK1	E. coli, λ	K-12	λ, pMB9, pBR322, pBR313	Exempt	Exempt
P1 + EK1	λ, E. coli	K-12	pBC16, Col E1, pSC101, pBR322	Exempt	Exempt

Appendix B--32

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P1 + EK1	E. coli	K-12	E. coli	Exempt	Exempt
P1 + EK1	E. coli	K-12	Col E1	Exempt	Exempt
P1 + EK1	E. coli, K-12	K-12	Col E1, pSGp120	Exempt	Exempt
P1 + EK1	E. coli	K-12	λgt, Col E1	Exempt	Exempt
P1 + EK1	E. coli, K-12	K-12	λ, Col E1, RSF 1010	Exempt	Exempt
P1 + EK1	E. coli	K-12	Col E1	Exempt	Exempt
P1 + EK1	E. coli	K-12	Col E1, λ	Exempt	Exempt
P1 + EK1	E. coli	K-12	Col E1, λ	Exempt	Exempt
P1 + EK1	T4	K-12 (HR28)	pBR322	Exempt	Exempt
P1 + EK1	E. coli, K-12	K-12	λ	Exempt	Exempt
P1	B. subtilis	B. subtilis	B. subtilis	Exempt	Exempt
P1 + EK1	E. coli, PML 21, λ	K-12	λ	Exempt	Exempt
P1 + EK1	E. coli, λ	K-12	E. coli, λ	Exempt	Exempt
P1 + EK1	lac transducing phage	K-12	Col E1	Exempt	Exempt
P1 + EK1	E. coli, pCSR604	K-12	pMB9	Exempt	Exempt

Appendix B--33

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P1 + EK1	E. coli plasmids	K-12	pSC101, pMB103, pMB9, pBR313	Exempt	Exempt
P1 + EK1	Col E1	K-12	pSC201, pMB9	Exempt	Exempt
P1 + EK1	Col E1	K-12	Col E1	Exempt	Exempt
P1 + EK1	K-12	K-12	Col E1	Exempt	Exempt
P1 + EK1	T2, T4, T6	K-12	Col E1	Exempt	Exempt
P1 + EK1	E. coli	E. coli	Col E1	Exempt	Exempt
P1 + EK1	E. coli	K-12	Col E1	Exempt	Exempt
P1 + EK1	K-12	SF8*	λ	Exempt	Exempt
P1 + EK1	K-12	C600*	λ, Col E1	Exempt	Exempt
P1 + EK1	E. coli	K-12	Col E1	Exempt	Exempt
P1 + EK1	Serratia marcescens	K-12	Col E1, pSC101	?Exempt	Exempt
P1 + EK1	T4	K-12	λ	Exempt	Exempt
P1 + EK1	T4	E. coli	λ, Col E1	Exempt	Exempt
P1 + EK1	λ	E. coli	pMB9	Exempt	Exempt
P1 + EK1	E. coli	E. coli	λ	Exempt	Exempt

Appendix B--34

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P1 + EK1	λ	K-12	Col E1	Exempt	Exempt
P1 + EK1	P4	K-12	Col E, pCRl	Exempt	Exempt
P1 + EK1	F plasmid	K-12	Col E1, pSC101	Exempt	Exempt
P1 + EK1	E. coli	K-12	pMB9, pBR314, pBR317, Col E1	Exempt	Exempt
P1 + EK1	pSM-1	K-12	Col E1	Exempt	Exempt
P1 + EK1	K-12	K-12	λ, CK 11, pSC101, Col E1, pML2, pMB9, pBR313, pAR322	Exempt	Exempt
P1 + EK1	λ, Mu	K-12	λ, Mu	Exempt	Exempt
P1 + EK1	TN5	K-12	Col E1	Exempt	Exempt
P1 + EK1	K-12	K-12	λ	Exempt	Exempt
P1 + EK1	K-12	K-12	Col E1, pSC101, pMB9, , pBR322, x174, T7	Exempt	Exempt

Appendix B--35

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P1 + EK1	x174	K-12	pMB9	Exempt	Exempt
P1 + EK1	B. subtilis	B. subtilis	B. subtilis, or S. aureus plasmids	Exempt	Exempt
P1 + EK1	E. coli	K-12	Col E1	Exempt	Exempt
P1 + EK1	F plasmid	K-12	Col E1	Exempt	Exempt
P1 + EK1	Caulobacter crescentus	K-12	pMB9, pBR322	Exempt	~~Exempt~~ P1 + EK1
P1 + EK1	E. coli	K-12	E. coli plasmids	Exempt	Exempt
P1 + EK1	T7	HB101*	pMB9	Exempt	Exempt
P1 + EK1	E. coli, K-12	K-12	Col E1	Exempt	Exempt

Current Containment Levels (P and EK)	Source of DNA	Host	Vector	RAC Revised Containment Level	NIH Revised Containment Level
P1 + EK1	Promoter-containing Bacteriophage E. coli, EcoRI	K-12	Col E1	Exempt	Exempt
P1 (NIH approved)	B. subtilis B. pumilus B. lichenformis	B. subtilis	pUB110, pC194, pPL10, pP1576	?Exempt	Exempt
P1 + EK1	Enterobacter aerogenes	K-12	pHR322, pHV317	?Exempt	Exempt

*Derivatives of E. coli K-12.

APPENDIX C

RESPONSES TO SOME COMMENTS ON THE NIH ENVIRONMENTAL IMPACT STATEMENT, BY PUBLIC WITNESSES AT THE DAC MEETING, DECEMBER 15-16, 1977

At the public meeting of the Advisory Committee to the Director, NIH, held at the National Institutes of Health in December 1977, witnesses representing environmental groups commented on the "Environmental Impact Statement on NIH Guidelines for Research Involving Recombinant DNA Molecules," published by NIH in October 1977. Some of the comments and responses by the Office of the Director, NIH, are summarized below.

Comment 1. "The environmental impact statement does not respond to the arguments that any gene, if expressed out of its normal context, has the potential for being 'harmful.' Appendix K59-62" (of the EIS).

Response. Page 23 of the EIS (section IV-C-1) states that the "stable insertion of DNA derived from a different species into a cell or virus (and thus the progeny thereof) may change certain properties of the host. The changes may be advantageous, detrimental, or neutral with regard to (*a*) the survival of the recipient species, (*b*) other forms of life that come in contact with the recipient, and (*c*) aspects of the nonliving environment. Current knowledge does not permit accurate assessment of whether such changes will be advantageous, detrimental, or neutral, and to what degree, when considering a particular recombinant DNA experiment." This statement and subsequent elaboration acknowledges the commentator's point—that any gene, if expressed out of its normal context, has a potential for doing harm. The EIS does not qualify here—does not limit itself to the effects of "harmful" genes, such as those coding for synthesis of a toxin. Section IV-C proceedes to discuss ways in which hazardous agents might be produced. Factors such as survival of the host cell, survival of vectors, and effects of hosts or vectors on other forms of life are considered. Moreover, the guidelines classify all DNA that might be manipulated, so that some measure of containment is required for any recombination. Thus "any gene" is covered.

Comment 2. "The EIS does not respond to the argument that while *E. coli* K-12 does not normally colonize the human colon, it is incorrect to infer that this property renders it harmless to humans. Appendix K62-64."

Response. The letter cited (Zimmerman, Environmental Defense Fund) argues that *E. coli* K-12's survival *per se* is not the issue—that genetic exchange is. The point is covered in section IV-C-1-b-(2) on page 29 of the EIS: "When a cell or virus dies, or comes close to or invades the tissue of another living organism, the re- combinant DNA may effectively enter a new cell. A hazardous situation * * * might ensue if foreign proteins were manufactured in this 'secondary' recipient" (et seq.). Further treatment of the issue is presented in section VI-C, page 76, under the heading "Transfer of Foreign DNA from *E. coli* K-12." The EIS does not infer that the demonstrated reluctance of *E. coli* K-12 to colonize the human intestine renders it entirely harmless. If that were so, there would be no guidelines as we know them, where experiments using this attenuated organism are classified over the whole range of containment possibilities.

Comment 3. "The possibility that an organism containing chimeric DNA could possess properties exhibited by neither the host nor the organism is not considered. No response to this in the EIS. Appendix K65."

Response. Section IV-C-1 of the EIS, pages 23-31, describes the ways in which recombinant DNA experiments might be hazardous. Under the subtitle "The effect of bacteria and viruses containing recombined DNA on other forms of life, "there are sections explaining basic mechanisms whereby a recipient microorganism might be altered with regard to its pathogenicity or toxicity as a result of a resident recombinant. "Foreign DNA inserted into a bacterial gene," it is stated, "might so alter the microbial cell's properties that it becomes harmful to other organisms. This might happen, for example, through a change in the growth rate and competitive advantage of the recipient microbial cell, resulting in increased virulence of a mildly pathogenic bacteria." (EIS, p. 29) The statement and those following it are broad enough to include the possibility that "an organism containing chimeric DNA could possess properties exhibited by neither the host nor the organism" (which probably means "neither the foreign donor nor the host organism"). In other words, the EIS has nowhere denied this possibility and, indeed, addressed it in a general way by considering the various mechanisms through which hazards might arise.

Comment 4. "There is no adequate response to the criticism that the publication of the EIS after the release of the guidelines negated the purpose of the National Environmental Policy Act, i.e., to secure broad public comments on the guidelines and the policy of expansion they assume. Appendix K31."

Response. The EIS on page 2 responds to the above criticism as follows: "Although NEPA assumes that such Federal actions will not be taken until the NEPA procedures are completed, the Director of NIH concluded that the public interest required immediate issuance of the guidelines, rather than deferral for the months that would be required for completion of the NEPA process. This was because experiments utilizing recombinant DNA technology were proceeding in various laboratories throughout the country with only general and purely voluntary restrictions." The statement is still valid, and it is difficult to see how the NIH might better have acted in the public interest. NIH has pointed out elsewhere that the release of guidelines was a positive act in the interest of public safety, not an act that could in any way be construed as negative or hazardous.

Comment 5. "No response or correction of the lack of 'classification system for pests and pathogens of plants and animals on the basis of their hazards to agriculture such as exists for etiologic agents of disease on the basis of their hazard to humans.'" Correspondence between Drs. John W. Littlefield and Peter Day, April 14, 1977.

Response. The EIS addresses the guidelines' treatment of plant and animal sources of DNA on page 120, first paragraph. It explains that the analysis of potential hazards in the EIS is given in a general way that is equally applicable to persons, animals, and plants. The section of the guidelines on plants has been expanded in the proposed revision. Specifications for plant host-vector systems provide safeguards in greenhouses, growth chambers, etc., that meet the standards for experiments involving other life forms. As to a classification system, a workshop on risk assessment of agricultural pathogens, comprising distinguished American plant pathologists, was held on March 20-21, 1978 (as announced on March 6 in the FEDERAL REGISTER) under the sponsorship of the U.S. Department of Agriculture, the National Science Foundation, and the National Institutes of Health. The report of this workshop constitutes appendix G. It was presented to the Recombinant Advisory Committee at its meeting on April 27-28, 1978, and was unanimously endorsed with certain minor amendments. The recommendations of the RAC have been adopted in the NIH Director's proposed revised guidelines (PRG-NIH) with additional minor amendments to sections dealing with the use of plants and plant pathogens in recombinant DNA research.

Comment 6. "No response to Dr. Peter Albersheim's concern over the use of the organism *Agrobacterium tumefaciens* in nitrogen-fixation work. Correspondence with Dr. Fredrickson,

July 10, 1977. *Comments* (peach-colored book) p.1."

Response. The letter to Dr. Fredrickson, July 10 (actually to Dr. Gartland, July 5, and received July 10) comments on the guidelines rather than the EIS. All letters received in response to the draft EIS are included in appendix K of the final EIS and are responded to on page 113-137 of the final EIS. Our response to Comment 5 above is also applicable to this comment—i.e., the EIS discusses plants on pages 23 and 120, and further discussion of this issue was held at the recent workshop on risk assessment of agricultural pathogens, which has led to changes in the PRG-NIH.

APPENDIX D

DISCUSSION OF THE "LIST OF EXCHANGERS" CONSTITUTING APPENDIX A TO THE GUIDELINES

Section I-E-4 of the Guidelines states that certain recombinant DNA molecules are exempt from the Guidelines. These are molecules that "consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such will be prepared and periodically revised by the Director, NIH, on the recommendation of the Re- combinant DNA Advisory Committee, after appropriate notice and opportunity for public comment. Certain classes are exempt as of publication of these Revised Guidelines. The list is in Appendix A."

The natural transfer of genes between bacteria occurs by transduction (bacterial virus-mediated), transformation (uptake of isolated DNA by a bacterial cell), or conjugation (plasmid-mediated transfer of genes between bacteria, requiring cell-to-cell contact). A reasonable generalization is that virtually all closely related species of bacteria can exchange genes by transduction and transformation, the former limited by the relatively narrow host-range of transducing bacteriophage and the latter by the requirement, in the case of chromosomal DNA, for homology of DNA in most recombination events. Conjugal mating with exchange of DNA can occur between virtually all Gram-negative bacteria, including naturally occurring soil and intestinal species, when mediated by a plasmid of broad host-range (for example, the Inc P-1 group plasmids). Recently, conjugal mating has also been shown to occur between strains of certain species of *Streptococcus*, a Gram-positive organism (for example, *Streptococcus faecalis*). To date, however, conjugal mating has not been demonstrated between Gram-negative and Gram-positive bacteria.

The relatedness of different microbial species can be estimated by determining the extent of DNA homology between them or by studying the properties of different microorganisms in genetic crosses. As a general rule, organisms that show considerable homology of their nucleotide sequences under a standard set of experimental conditions have the capacity to mutually integrate chromosomal genes. For example, in the case of the *Enterobacteriaceae* family of bacteria (includes *Escherichia coli* K-12), there is both extensive DNA-DNA homology (*1*) and chromosomal gene exchange, (*2*) with a reasonable correlation between the degree of DNA-DNA homology and the capacity to mutually integrate chromosomal genes.

Genetic relatedness, as indicated by a high level of DNA-DNA homology between different microorganisms, is not, however, an absolute requirement for the exchange of chromosomal genes between bacteria. In fact, chromosomal gene transfer among diverse members of the Gram-negative group of bacteria has been demonstrated where the microorganisms involved show little or no DNA-DNA homology. In these cases the exchange of chromosomal genes is promoted by a broad-host-range plasmid of the Inc P-1 incompatibility type. These plasmids mobilize the chromosomes of a wide variety of Gram-negative bacteria, incorporate segments of these chromosomes, and are capable of establishing themselves along with covalently-linked chromosomal genes in a wide range of Gram-negative bacteria. (*3*)

Appendix A to the Guidelines contains a list of documented cases of chromosomal gene exchange between a variety of bacteria and *E. coli* K-12 where the gene exchange is promoted by Inc P-1 or other plasmids. The first ten entries in this table are members of the *Enterobacteriaceae* family of bacteria. The bacterial species in this family not only show chromosomal gene exchange but, as indicated above, exhibit extensive DNA-DNA homology. References listed at the end of this Appendix (*4*) provide documentation for the entries in Appendix A to the Guidelines. All entries in Appendix A exhibit R-prime transfer (R plasmid carrying chromosomal genes) to *E. coli* K-12 mediated by the Inc P-1 or other plasmids.

The Recombinant DNA Advisory Committee at its April 27-28, 1978, meeting proposed three possible alternative lists for consideration by the Director, NIH, to become Appendix A to the Guidelines.

The first list with its criteria and entries was as follows:

The following organisms of the *Enterobacteriaecae* family exhibit chro- mosomal DNA relatedness (20 percent or more homology of DNA of various pairs tested) and genetic recombination or R-prime (R plasmid carrying chromosomal genes) transfer to *E. coli* K-12 mediated by the Inc P-1 plasmids.

All species of the following genera:

1. *Escherichia* (including *E. coli* K-12)
2. *Shigellae*
3. *Salmonella*
4. *Enterobacter*
5. *Arizona*
6. *Citrobacter*
7. *Klebsiellae*

In addition, the following species:

1. *Erwinia amylovora*
2. *Erwinia dissolvens*
3. *Erwinia minipressuralis*
4. *Serratia marcescens*
5. *Levinea malonatica*
6. *Levinea amalonatica*

The second list proposed by the RAC included all the members of the first list but added additional members. The criteria used are that all the members of the list exhibit R-prime transfer (R plasmid carrying chromosomal genes) to *E. coli* K-12 mediated by the IncP-1 or other plasmids. This is the list which (which certain deletions) was selected by the Director, NIH to become Appendix A to PRG-NIH. The references supporting the entries to this list are give (*4*) at the end of this Appendix.

The third list included all the members of the second list but adds additional members. This list, with its criteria and entries, was as follows:

The following genera and/or species possess R plasmids (including R plasmids of the IncP-1 group) transferable to *E. coli* K-12.

1. All members of the *Enterobacteriaceae* family
2. *Vibrio* species (except *Vibrio para- hemolyticus*)
3. *Pseudomonas* species
4. *Rhizobium* species
5. *Acinetobacter calcoaceticus*
6. *Agrobacterium tumefaciens*
7. *Rhodopseudomonas sphaeroides*
8. *Caulobacter crescentus*
9. *Proteus* species
10. *Achromobacter* species
11. *Aeromonas salmonicida*
12. *Alcaligenes faecalis*
13. *Bordetella bronchiseptica*
14. *Myxococcus xanthus*
15. *Neisseria gonorrhoeae*
16. *Pastuerella hemolytica*
17. *Pastuerella multocida*
18. *Yersinia* species (excludes *Y pestis*, since it is a Class 3 agent)
19. *Xanthomonas* species

In addition, recombinant DNA experiments between *H influenzae* and *H. parainfluenzae* are exempt on the basis of extensive DNA homology.

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APPENDIX E

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE,
PUBLIC HEALTH SERVICE,
NATIONAL INSTITUTES OF HEALTH,
Bethesda, Md. March 16, 1978.

Dr. DONALD S. FREDRICKSON,
Director, National Institutes of Health, 9000 Rockville Pike, Bethesda, Md.

DEAR Dr. FREDRICKSON: On January 26-28, 1978, a joint U.S.-EMBO Workshop to Assess Risks for Recombinant DNA Experiments Involving the Genomes of Animal, Plant, and Insect Viruses was held in Ascot, England. The workshop was sponsored by the NIH at your request, in response to discussions concerning viruses at the Director's Advisory Committee meeting of December 1977. The workshop was attended by 27 scientists from the United States, the United Kingdom, West Germany, Finland, France, Sweden, and Switzerland. The participants were invited because of their scientific expertise and not as representatives of any government or of any policymaking group.

The primary purpose of the meeting was to conduct a scientific and technical analysis of possible risks associated with cloning eukaryotic viral DNA segments in *E. coli* K-12 host-vector systems and with the use of eukaryotic viruses as cloning vectors in animal, plant, and insect systems. In addition, there were general discussions of the possible importance of recombinant DNA technology for the solution of problems in basic and applied virology and of the classification of viruses with respect to the hazard that laboratory research with them might pose to the laboratory worker or to the community.

A report of the discussions and conclusions of the workshop is transmitted to you along with this letter. A draft of this report was sent to the members for comment and revision, and this final version is based on the replies of all the participants. In view of the favorable responses, we feel that this report will receive the support of virologists in general. We hope that it will be useful to you and the various national committees that are considering containment levels for this type of recombinant DNA research.

Since this is the report of an international group, we generally avoided reference to any particular set of containment conditions. Rather, we anticipate that the various national committees will use this report as the scientific basis for setting the containment conditions they feel appropriate.

Sincerely,

MALCOLM A. MARTIN,
Laboratory of Biology of Viruses, NIAID.
WALLACE P. ROWE,
Laboratory of Viral Diseases, NIAID.
JOHN TOOZE,
Executive Secretary, European Molecular Biology Organization, Heidelberg.
Co-Chairmen.

REPORT OF U.S.-EMBO WORKSHOP TO ASSESS RISKS FOR RECOMBINANT DNA EXPERIMENTS INVOLVING THE GENOMES OF ANIMAL, PLANT, AND INSECT VIRUSES

This is the report of a joint U.S.-EMBO workshop held in Ascot, England, January 27-29, 1978, which was convened to discuss the possible risks of recombinant DNA experiments involving the DNAs of animal, plant, and insect viruses. The 27 scientists in attendance (see attached roster) had expertise in clinical infectious disease; public health, medical and diagnostic virology; the biology of virus infection; biochemical virology; and plant, insect, and veterinary viruses. Five of the participants are actively engaged in recombinant DNA experimentation. A consensus statement of the discussions in the areas of pathogenesis and epidemiology of viral diseases, potential benefits of recombinant DNA experiments involving eukaryotic viral DNA, viral hazard classifications, and cloning in prokaryotic and eukaryotic systems is presented below. The group's conclusions, with respect to possible risks of recombinant DNA experiments involving viruses are based on the best available scientific data derived from publications, knowledge of current activities in the field of virology, and first-hand experience in the virology laboratory.

Introduction

Viral disease is a complex process that involves a series of critical steps; these include entry of the virus particle into the host, infection of specific cells at the portal of entry, replication of the virus in the infected cells, and usually, the spread of the progeny virus particles within the infected host to other susceptible cells. Depending upon the nature of the particular viral agent, the deleterious effects for the host, if any, may result from cytolytic activity, cellular transformation, chronic cellular dysfunction, or the provocation of an injurious immunological response. Viruses contain 5 to 150 or more genes and their coordinated functioning is required for viral growth and, consequently, for survival of the virus in nature. Even though we do not generally understand the precise role of each viral gene product, it seems clear that viral infection and disease production requires proper functioning of most, if not all, viral genes and, in general, is not a consequence of any single viral gene product. In the case of oncogenic papovaviruses, transforming retro- viruses and possibly adenoviruses, individual viral genes are thought to be responsible for the transforming properties of the virus.

Recombinant DNA experiments have already yielded new information about the structure and control of expression of genes in higher organisms that could not have been obtained by conventional techniques. DNA cloning provides unparalleled opportunities to explore the basic biology of animal and plant viruses. Virologists will be able to probe more deeply into the control of viral gene expression and

discover phenomena of general cell biological significance; techniques will be more readily available to elucidate the sequence of viral nucleic acids, to shed light on the role of viral gene products in pathogenicity, and eventually, to understand the molecular biology of animal and plant viruses to the extent that some bacteriophages are now understood. It seems apparent that this new information will lead to a deeper understanding of viral diseases and to new ways of combating them. In the immediate future the ability to obtain useful amounts of pure viral genomes and subgenomic fragments that cannot be obtained by other means will provide scientists and physicians with invaluable and inexpensive diagnostic protein and nucleic acid reagents. In the more distant future it should be possible to use gene cloning techniques to obtain large amounts of viral proteins; one practical benefit from such developments might well be effective and safe vaccines for control of diseases caused by hepatitis viruses, herpesviruses and influenza viruses and many other viruses, both known and, as yet, unknown.

In addition to being able to clone viral genes in bacteria we are now able to envisage using certain animal viruses as vectors for the propagation of foreign genes in animal cells; a similar system for exploiting a plant virus, cauliflower mosaic virus, to clone foreign genes in plant cells may shortly become available. The chief importance of animal and plant virus vectors is that they can be used to carry genes into cells in which they may be fully expressed as well as propagated. By using specifically designed viral vectors it may eventually prove possible to deliver a specific gene to specific target cells; such techniques have obvious medical, economic, and agricultural applications but their realization will depend upon a great deal of basic research.

VIRAL CLASSIFICATION SYSTEMS AND RECOMBINANT DNA EXPERIMENTATION

The group extensively discussed the current safety procedures for holding and handling in the laboratory certain animal viruses, in particular those likely to be used in cloning experiments in the foreseeable future, either as vectors or as the sources of the nucleic acids to be cloned. Inevitably the recommended safety measures for using animal (and plant) viruses in research vary from country to country. In the context of recombinant DNA research, which involves a novel set of circumstances, none of the available classifications of viruses according to the risks they pose is entirely satisfactory. A list of animal viruses was therefore prepared and the viruses were ranked according to their known hazard on the basis of: (a) The severity of human disease that they can cause, particularly in persons exposed in the laboratory; (b) their potential for infecting laboratory workers; (c) the risk that a laboratory infection might result in spread to the community; and (d) the impact such spread might have on the community or environment (table 1). In this list four bacteria and one rickettsial agent with different pathogenic potentials have also been included as a frame of reference for the 22 viruses identified. Because of time constraints, many animal viruses, particularly those of agricultural and veterinary importance, were not included in the table. Although not comprehensive, the list contains most of those animal viruses that have been previously mentioned in the context of recombinant DNA experiments.

This list can also serve the very useful function of a reference scale, familiar to both microbiologists and clinicians, for expressing the degree of concern that a given conjectural hazard may engender, by comparison to a known biohazard.

CLONING VIRAL DNA'S IN E. COLI K-12

The cloning of viral DNA's and cDNA's in *E. coli* K-12 using EK1 and EK2 plasmid and lambda phage vectors was discussed in light of the conclusions of the Falmouth meeting that *E. coli* K-12 is not pathogenic and does not efficiently colonize the vertebrate digestive tract (Gorbach, 1978). Not for want of trying, the participants were unable to envisage a sequence of events which could occur with significant probability that would allow *E. coli* carrying either whole DNA genomes of certain viruses or subgenomic fragments of virtually any virus to lead to disease. The question was also raised as to whether or not, in the extremely remote possibility that all of the biological and physical containment barriers broke down, intestinal bacteria carrying cloned whole viral genomes might bypass the natural barriers to infection by the virus particle. As summarized in the following section, the group concluded that the probability that K-12 organisms carrying viral DNA inserts could represent a significant hazard to the community was so small as to be of no practical consequence.

Risk assessment analysis for cloning viral DNA in E. coli K-12.[1] The following is a summary of the workshop discussions dealing with possible mechanisms of risk resulting from the cloning of genetic material of eukaryotic viruses (i.e., viruses of animals, plants, or lower eukaryotes) in *E. coli* vector systems.

We started with the extremely unlikely "worst case" assumption that a recombinant molecule containing eukaryotic viral genome sequences has in some way become established in wild type *E. coli* and has thereby become disseminated throughout the bowel flora of vertebrates. Given this hypothetical set of conditions, what consequences could be envisaged? For the purposes of analysis the discussions focused on two issues.

First: The mechanisms by which the viral nucleic acid might gain access to cells of the host; and

Second: The nature of the inserted viral sequences.

Access of the viral genome to cells of the vertebrate host might conceivably result from virus particles formed within the bacterium or from release of viral nucleic acid into the proximity of, or into, host cells; this could occur either in the intestine or at the site of extraintestinal *E. coli* infection.

Production of infectious virus particles by bacteria carrying the recombinant DNA molecules was considered to be virtually impossible, regardless of the completeness of the viral genome or the nature of the eukaryotic virus from which it was derived. The basis for this high degree of assurance is in large part our great understanding of the molecular biology of virus replication. The nature of regulation of gene expression in prokaryotes is clearly different from that in eukaryotes as particularly exemplified by the absence in prokaryotes of RNA splicing mechanisms (Berget el al., 1977; Aloni et al., 1977; Lavi and Groner, 1977; Mellon and Duesberg, 1977; Chow et al., 1977; Klessig, 1977; Dunn and Hassell, 1977), RNA capping (Moore, 1966; Stavis and August, 1970; Blattner and Dahlberg, 1972; Maizels, 1973; Wei and Moss, 1975; Furuichi et al., 1975a; Keith and Fraenkel-Conrat, 1975; Abraham et al., 1975; Furuichi et al., 1975b; Dubin and Taylor, 1975; Perry and Scherrer, 1975; Moss and Koczot, 1976) and differences in messenger RNA biogenesis, polyadenylation (Kates and Beeson, 1970; Darnell et al., 1971; Mendecki et al., 1972; Philipson et al., 1971; Weinberg et al., 1972; Ehrenfeld and Summers, 1972; Pridgen and Kingsbury, 1972), and ribosomal binding sites (Shine and Dalgarno, 1974; Steitz and Jakes, 1975; Hagenbuchle et al., 1978). The expression of animal viral mRNA in *E. coli* translation systems is not accurate; in one well studied system, poliovirus, internal initiation signals are read which result in premature chain termination (Rekosh et al., 1970). Thus neither the synthesis of proper mRNA nor its translation into viral protein is likely to occur in *E. coli*.

[1] This portion of the report was prepared subsequent to the U.S.-EMBO workshop by Drs. Martin and Rowe.

Virus replication requires the regulated and coordinated function of multiple enzyme systems derived from both the host and the viral genomes; it seems most unlikely that any prokaryote contains the complement of enzymes required for the synthesis of an infectious animal virus. Animal viruses have evolved to be adapted to replication in eukaryotic cells; except for the instances where they have coevolved to replicate in insect vectors, animal and plant viruses show a high degree of specificity for cells of a particular class or species of host cell or even a particular differentiated cell type. Further, there is no verified example of the replication of a virus of eukaryotes in bacteria, or conversely, the replication of a bacterial virus in any eukaryote. Consequently, since it was not considered possible for recombinant DNA containing a viral chromosome to produce intact virus particles in *E. coli*, the group felt secure in focusing on models involving host cell exposure to viral nucleic acid rather than viral particles.

In a model in which recombinant DNA-containing organisms are confined to the lumen of the intestine, the possibility of viral RNA or DNA gaining access to mucosal cells is extremely remote. First: The large amounts of nucleases in the intestinal contents would rapidly destroy the recombinant molecules (Maturin and Curtiss, 1977). Second: The efficiency of infection of cells by viral nucleic acid molecules, even in the presence of chemical potentiators, is extremely low, both in vitro and in vivo (Ellem and Colter, 1961; Amstey and Parkman, 1966; McCutchan and Pagano, 1968; Graham and van der Eb, 1973; Israel et al., 1978a). Third: Since animal experiments indicate that polyoma viral DNA cannot initiate infection when administered by the oral or nasal routes (Israel et al., 1978a), it is likely that nucleic acids cannot infect across mucous membranes.

Consequently, the only model to which serious attention must be directed is one in which the hypothetical bacterium carrying the viral recombinant DNA gains access to the body tissues. Two cases can be considered. With minor transgressions of the intestinal mucosa that allow brief penetration of organisms into the intestinal wall, lymphatics, or the protal circulation, the bacteria would be ingested by phagocytes. In phagocytes, bacteria are lysed and their nucleic acid is released in the nuclease-rich lysosomes; the effectiveness of the lysosomal enzymes virtuall guarantees that no nucleic acid could survive to cause infection (Bensch et al., 1964; Carrara and Bernardi [1968]; Arsenis et al., 1970).

The second case, extraintestinal infection such as urinary tract or surgical wound infection, deserves serious consideration, but it must be pointed our that this would occur in only a fraction of individuals. In this context the consequences of the infection would be a function of the nature of the viral DNA inserted in the recombinant DNA molecule. We considered the following classes of DNA inserts: (1) Subgenomic segments of nononcogenic viruses; (2) transforming segments of oncogenic viruses; (3) cDNA prepared from the genome of segmented RNA viruses; (4) cDNA copies containing the complete viral genome of nonsegmented RNA viruses and (5) complete DNA viral genomes. The workshop participants reached the conclusion that viral inserts should be thought in terms of three classes of risk: Those for which there was no risk of a harmful outcome, and those for which a possibility of harm, however remote, could be envisioned. The latter were then divided into those scenarios which may well in reality be impossible, and those which are felt to be possible.

Those inserts for which the group could not construct any realistic harmful scenario were: (1) Subgenomic segments of nontransforming RNA or DNA viruses; (2) cDNA copies from RNA viruses with segmented viral genomes and (3) cDNA's of the complete genome of negative strand RNA viruses.

(1) Subgenomic segments (meaning small portions of the viral genome lacking genes needed for replication of the virus) of nontransforming viruses are considered to be harmless because of the absence of any known example of an individual viral encoded protein which can act exogenously on cells. With the exception of some glycoproteins when added to cell cultures in high concentrations (Scheid and Choppin, 1974; McSharry and Choppin, 1978), viral proteins do not induce cell damage from without.

(2) Reverse transcripts of RNA viruses with segmented genomes cannot be envisaged as carrying any risk of producing infectious virus even when the cDNA is made from unfractionated nucleic acid preparations. It would be virtually impossible to ligate together a complete DNA copy, and if this ever did occur, we cannot envisage any way that the proper length RNA genome segments could be transcribed therefrom.

(3) Cloning of reverse transcripts of negative strand RNA viruses is viewed as being free of risk. With these viruses, the process of viral mRNA and genomic RNA synthesis is complex, and the Workshop participants could not envisage a set of circumstances in which RNA segments, transcribed from a DNA template, could eventuate in the synthesis of progeny virus. This is based on the fact that the nucleic acid of negative strand RNA viruses (either the plus or minus strand) has never been shown to be infectious for cells (Kingsbury, 1966; Baltimore et al., 1970; Wagner, 1975) presumably because of their unique molecular biology. Infection by negative strand viruses requires the activity of the virion associated transcriptase (Baltimore et al., 1970; Cormack et al., 1971; Szilagyi and Pringle, 1972); this enzyme catalyzes the synthesis of multiple (segmented) functional plus strand mRNA molecules from the input minus strand (Bratt and Robinson, 1967; Huang et al., 1970; Mudd and Summers, 1970; East and Kingsbury, 1971; Weiss and Bratt, 1976; Freeman et al., 1977). A polypeptide specified by one of these mRNAs then modifies the virion transcriptase to function as a replicase mediating the synthesis of a complete unsegmented plus strand of RNA, the template for synthesis of progeny minus stand RNA molecules. The minus strand has no messenger function (Huang et al., 1970; Grubman and Summers, 1973; Kingsbury, 1973; Morrison et al., 1974). Thus, to initiate the infectious process both a full length RNA transcript (to serve as template) and the segmented plus strand transcripts, capped for function as mRNA, would be required. This would necessitate either the transcription of both DNA strands by cellular RNA polymerase or the synthesis of a full length transcript as well as properly terminated, segmented transcripts from the minus strand DNA. These are obviously extremely unlikely events.

There were two classes in which it was considered that a risk scenario might be constructed but which might indeed be impossible; these involve the cloning of the transforming segments of DNA viruses or of transforming retroviruses, and the cloning of complete reverse transcripts of plus-strand RNA viruses. The model involving subgenomic, transforming segments postulates release of recombinant DNA molecules by lysis of *E. coli* in the tissues, and integration of the transforming gene segment into the DNA of host cells. While this eventuality cannot be ruled out, it was considered to have a very low probability in view of the inefficiency of transforming cells with viral nucleic acid (Aaronson and Martin, 1970; Graham et al., 1974; Abrahams et al., 1975) and the fact that integration of transforming DNA would occur in only a few cells in any one individual and, in the presence of competent immuno- surveillance, would be most unlikely to result in a tumor (Habel, 1961; Sjogren, 1964; Sjogren et al., 1967; Costa et al., 1977). Transplanta-

tion studies with primary tumors and transformed tissue culture cells indicate that large numbers of cells are required to initiate tumor growth; in general, only after adaptation effected by long passage in animals do small numbers of cells produce tumors (Sjogren, 1964; Klein, 1975). In this regard, it should be noted that there is overwhelming evidence that humans are highly effective in averting tumorigenesis by DNA viruses. Man, and many other vertebrates as well, are infected repeatedly with DNA viruses (e.g., papovaviruses and adenoviruses) that contain the transforming regions discussed above (Huebner et al., 1954; Hilleman, 1957; Shah et al., 1973; Padgett and Walker, 1973; Brown et al., 1975; Shah and Nathanson, 1976). Some of these viruses are even known to induce tumors in laboratory rodents, yet, despite intensive search, none of these has been reproducibly associated with the etiology of any malignancy in humans (Mackey et al., 1976; Fiori and DiMayorca, 1976; Wold et al., 1978; Israel et al., 1978b).

The risk scenario whereby a cDNA transcript of a positive strand RNA virus leads to infection of the host was considered to have a finite but low probability. In this model, extraintestinal infection by the bacterium carrying the recombinant DNA results in the recombinant DNA molecule, or plus strand RNA transcripts thereof, entering into host cells with resultant productive viral infection. Neither carries a risk if the cDNA insert is not a complete copy of the RNA genome. Unless a deliberate attempt is made to select full-length cDNA molecules for ligation to prokaryotic DNA vectors, the vast majority of recombinant DNA molecules will contain only a segment of viral specific DNA and, as a consequence, the likelihood of cloning the whole genome will be low.

The major block to synthesis of infectious RNA from the recombinant DNA molecule either in the prokaryotic or eukaryotic cell would be the inaccurate initiation of transcription. There is no reason to believe that the 5′ terminus of the viral genome would generate a binding site for either prokaryotic or eukaryotic RNA polymerases following reverse transcription into DNA. Rather, RNA synthesis would be initiated either:

(1) Internally, by a random initiation process which would yield noninfectious molecules;

(2) Upstream, either at a prokaryotic promoter or at a random initiation site, in the eukaryotic cell which would generate a leader sequence lacking the appropriate recognition signals for binding to eukaryotic ribosomes (Shine and Dalgarno, 1974; Steitz and Jakes, 1975; Hagenbuchle et al., 1978). To produce infectious RNA from such a primary transcript, a site-specific cleavage would have to occur;

(3) At the 5′ terminus by random initiation, but this undoubtedly would be an extremely rare event.

Any full-length plus strand transcripts synthesized in *E. coli* would have to escape degradation by extracellular ribonucleases following their release from bacteria and then initiate an infection in a sensitive cell, which, under the best conditions in the laboratory, is an extremely inefficient process (Ellem and Colter, 1961).

Further studies of this type of viral DNA insert would clearly be desirable.

The cloning of the complete genome of DNA viruses (including proviral forms of retroviruses) provides a scenario felt to carry a finite probability of risk, but this too is considered extremely low. Since viral DNA molecules are generally infectious, any situation in which the complete viral genome, without deletion or substitution, is excised from the recombinant molecule could conceivably lead to infection. In particular, circular DNA genomes, cleaved with a single cut restriction enzyme and ligated to a prokaryotic vector, provide such a condition; however, for accurate excision one must postulate the unlikely eventuality of the recombinant molecule being brought in contact with the same restriction enzyme used during the insertion of the DNA segment into the vector. Complete viral genomes could also be generated from a recombinant molecule in the case of oligomers of viral DNA inserts; a complete copy of the viral DNA could then be generated by intramolecular recombination. Full length linear DNA molecules inserted into a vector by oligo dA-oligo dT tailing or by addition of other linker molecules would be unlikely to excise accurately terminating genomes.

Given that it is conceptually possible to generate infectious molecules from recombinant DNA containing the complete genome of DNA viruses, it is important to assess the factors that would result in such infectious DNA causing disease. The probability that the excised molecules would infect host cells is of course relatively low in view of the inefficiency of infection by naked DNA (Ito, 1960; Burnett and Harrington, 1968; McCutchan and Pagano, 1968; Mayne et al., 1971; Graham and van der Eb, 1973; Sol and van der Noordaa, 1977). Another important factor in such a scenario is the susceptibility of neighboring cells to the virions produced by a cell infected with the infectious DNA. If the surrounding cells were not sensitive to the virus itself, the initially infected cell, producing virions, could not spread this infection to other cells or tissues; in the absence of virus spread there would be no risk other than the possible transformation of a small number of cells, in the case of oncogenic viruses, as discussed above. Only if the host was sensitive to the virus would spread occur; this would not be different from infection with the virus itself and would not generally represent a unique biohazard. Futher study of this type of viral DNA insert would be extremely useful.

Recapitulation. It should be noted that these model scenarios were not constructed from an anthropocentric viewpoint, but apply generally to any model in which vertebrates are colonized by the *E. coli* carrying the recombinant molecule.

To recapitulate, even assuming a worst case situation, this analysis leads us to conclude that cloning the subgenomic segments of nononcogenic viruses, the complete genome of negative strand RNA viruses, and any part of the genome of segmented viruses carries no risk of generating a biohazard. Second: It is possible to construct conceivable but extremely unlikely sets of circumstances resulting in a biohazard from the cloning of the transforming segment of oncogenic viruses. And, third: It is possible to envisage feasible biohazard scenarios from cloning of the complete genome of DNA viruses or the entire genome of plus strand RNA viruses but even these carry little possibility of risk. When this analysis is combined with the immense unlikelihood of generating this worst case scenario in the first place, given good laboratory practice and the safety inherent in use of approved *E. coli* K-12 host-vector systems (Gorbach, 1978), the group felt strongly justified in concluding from available scientific information that viral genomes or fragments thereof, cloned in *E. coli* K-12 using approved plasmid or phage vectors pose no more risk than work with the infectious virus or its nucleic acid and in most, if not all cases, clearly present less risk. In fact, the Workshop participants agreed that cloning of viral DNA in *E. coli* K-12 may provide a unique opportunity to study with greatly reduced risks the biology of extremely pathogenic and virulent viruses.

The group also agreed that the cloning of cDNA copies of viroids in *E. coli* K-12 should be postponed until more information is available about their molecular and cellular biology.

Recommendation. Based on these considerations the participants of the U.S.-EMBO Workshop concluded that the use of P2 (NIH guidelines) or CI (Williams report) containment measures, in conjunction with an EK1 host-vector system should provide adequate containment for cloning any viral genome or fragment thereof and recommended this as the minimum con-

tainment levels for recombinant DNA experiments involving eukaryotic viral DNA inserts. However, if the virus itself must be handled at higher levels of physical containment it seems prudent at the present time to use the more stringent containment conditions. It was emphasized that containment practices must include adequate training and the use of high quality microbiological technique.

EXPERIMENTS WITH EUKARYOTIC HOST-VECTORS

1. Vertebrate host-vector systems in which viral DNA vectors are used to propagate other DNA segments.

The workshop participants endorsed the portion of the NIH guidelines that describes the features required for an animal virus to be used as a cloning vector (i.e., the first portion of section III.B.3.a. of the draft version, revised NIH guidelines for recombinant DNA research). The following is our recommended version of this section:

Because this work will be done almost exclusively in tissue culture cells, which have no capacity for propagation outside the laboratory, the primary focus for containment is the vector; it should be pointed out that the risk of laboratory acquired infection as a consequence of tissue culture manipulations is very low. Given good microbiological practices, the most likely mode of escape of recombinant DNAs from a physically contained laboratory is carriage by an infected human; thus the vector with an inserted DNA segment should have little or no ability to replicate or spread in humans. Further, a recombinant virus should not inadvertently pose a threat to any species.

For use as a vector in a vertebrate host cell system, an animal viral DNA molecule ideally should display the following properties:

(a) It should not consist of the whole genome of any agent that is infectious for humans or that replicates to a significant extent in human cells in tissue culture.

(b) It should be derived from a virus whose epidemiological behavior and biological properties are well understood.

(c) Its functional anatomy should be known—that is, there should be a clear idea of the location within the molecule of:

(i) The sites at which DNA synthesis originates and terminates;

(ii) The sites that are cleaved by restriction endonucleases;

(iii) The template regions for the major gene products.

(d) It should be defective when carrying an inserted DNA segment; that is, propagation of the recombinant DNA as a virus must be dependent upon the presence of a complementing helper genome. In almost all cases this condition would be achieved automatically by the manipulations used to construct and propagate the recombinants. In addition, the amount of DNA encapsidated in the particles of most animal viruses is defined within fairly close limits. The insertion of sizeable foreign DNA sequences, therefore, generally demands a compensatory deletion of viral sequences. It may be possible to introduce very short insertions (50–100 base pairs) without rendering the viral vector defective. In such a situation, the requirement that the viral vector be defective is not necessary.

If possible the helper virus genome should:

(i) Be integrated into the genome of a stable line of host cells (a situation that would effectively limit the growth of the vector recombinant to such cell lines); or

(ii) Consist of a defective genome, or an appropriate conditional lethal mutant virus, making vector and helper dependent upon each other for propagation.

However, neither of these stipulations is a requirement.

The group discussed at length the possibility that use of eukaryotic vectors under these conditions could pose a threat to the community or environment, but could not envisage a plausible set of circumstances whereby a risk to the community could develop. Given the extent of the genetic deletion required to satisfy the conditions stated above and in table 2, and the constraints that encapsidation places on the size of an inserted gene segment, the workshop participants saw no way that the experiments could generate a competent virus containing new genetic information sufficient to code for a functional gene product. The group tried to conceive of ways by which a recombinantional event between the defective recombinant genome and the helper genome or the genome of an indigenous virus in an exposed laboratory worker might generate nondefective viral recombinants, but as mentioned, was unable to identify any.

The possibility that a defective recombinant genome might be maintained in nature through complementation by a helper virus was considered too unlikely to be a cause for concern, given the absence of any precedent in animal virology. The group considered the adeno-associated viruses, which are defective parvoviruses maintained in nature, to be a unique case in that they are known to integrate into host cells and to have at least 33 different potential helpers (human adenoviruses) available.

Having considered the use of the genomes of different DNA animal viruses to propagate DNA sequences from different sources, the group proposed the following recommendations:

TABLE 2

Viral Vector DNA	Precaution Level Depending on Source of Foreign DNA		
	prokaryotic	eukaryotic	viral
polyoma virus — all or part	L or M	L	CbC
SV40 — unconditionally defective by deletion of all or part of the sequences of any of the genes	L or M	M	CbC
adenoviruses 2 and 5 — incapacitated by deletion of at least two capsid genes	L or M	L	CbC
murine adenovirus strain FL — all or part[+]	L or M	L	CbC
Herpes simplex virus — incapacitated by a large deletion	CbC	CbC	CbC

L corresponds to a containment level approximately equivalent to P2.

M corresponds to a containment level approximately equivalent to P3.

Prokaryotic sequences that are known not to contain toxigenic genes may be cloned in *L* conditions: otherwise *M* conditions should be used.

+ Before this viral DNA can be used as a vector, further information is required about its host-range, particularly its interactions with cultured primate cells.

CbC these experiments should be assessed by the appropriate committees on a "Case by Case" (CbC) basis.

The group recommended that further work on the biological properties of recombinants formed between the genomes of two animal viruses should be carried out as a matter of some importance. The workshop participants discussed the possibility that new viral agents might be created with novel biological properties (e.g. host range, tissue tropism, pathogenicity) not found in either parent. Several model experiments were proposed to test this possibility. The group recommended that research in this area proceed cautiously with each case being considered on its individual merits and even if the model experiments suggest little cause for concern, continued careful surveillance of new recombinants should be maintained.

2. *Vertebrate host-vector systems in which recombinant DNAs are used to transform cells.* In these types of experiments viral DNAs carrying a foreign DNA segment will be used to transform cells in culture and in the process, integrate the recombinant DNA into the host cell chromosome. Some transformation systems are nonpermissive for progeny virus production and pose no possibility of producing laboratory infections. Other transformation systems are semi-permissive and, in addition to the appearance of transformed cells, allow the production of low titers of infectious virus.

When recombinant DNAs are used to transform cells which do not yield significant quantities of infectious virus (e.g. SV40 in murine cells, polyoma virus in rat or hamster cells, adenovirus 2 and 5 in rat cells) *L* conditions are generally sufficient; for viruses which do not infect humans, there need be no requirement that the vector be disabled. *M* conditions should be used if the system is semipermissive (i.e., virus is produced, in low titer) and the virus is capable of infecting humans (e.g. SV40 in human cell cultures). It was agreed that the DNA to be cloned must not be derived from another animal virus.

The use of viral genes as selective markers offers exciting and important possibilities for experiments involving cloning in eukaryotic cells. Possibly the best of such markers would be the herpes simplex virus thymidine kinase gene. We recommend that special emphasis be given to cloning in prokaryotic systems a small fragment (<5KB) of herpes viral DNA which contains the sequences of this gene. Once available, the purified segment could be ligated to any chosen piece of DNA and cells transformed by the recombinant could easily be selected by virtue of the presence of thymidine kinase.

3. *Baculoviruses as vectors.* The group discussed the use of baculoviruses (large DNA-containing insect viruses) for cloning genes in invertebrate cells and concluded that our knowledge of the molecular and cellular biology of these viruses is too limited to allow any general recommendations. Proposals to use these viruses should be considered case by case. The group also recommended, however, that because of the potential exploitation of these viruses as biological pesticides (two have already been licensed for this purpose) and as vectors in recombinant DNA experimentation, high priority should be given to studies of their basic virology, genetics, and molecular biology.

4. *Cauliflower Mosaic Virus as a vector for cloning genes in plant cells.* The only known plant viruses which could serve as vectors for cloning genes in plants and plant cell protoplasts are Cauliflower Mosaic Virus (CaMV) and its close relatives, which have relaxed circular double stranded DNA genomes with a molecular weight of 5×10^6. The genomes of these viruses are not known to integrate into host chromosomes, or to pick up cellular genes. CaMV is spread in nature by aphids, in which it survives for a few hours. Spontaneous mutants of CaMV that are not transmitted by aphids arise frequently; these mutants fail to make a transmission factor essential for aphid transmission.

The viruses in the CaMV group have narrow host ranges and are relatively difficult to transmit mechanically to plants. For this reason, they are most unlikely to be accidentally transmitted from spillage of purified preparations of the virus.

The workshop participants recommended that for use as a vector with intact plants, a strain should be selected which is not transmitted by aphids The ability to produce local lesions in an appropriate host would be an advantage in maintaining the integrity of the strain. The plants should be grown in either a greenhouse or plant growth cabinet which is insect-proof Soil, plant pots and unwanted infected plant materials should be removed from the greenhouse or cabinet in sealed insect proof containers and sterilized. It is not necessary to sterilize run-off water from the infected plants as this is not a plausible route for secondary infections. Infected plant materials to be used for further research, which have to be removed from the greenhouse or cabinet, should be maintained under insect proof conditions. These measures provide an entirely adequate degree of containment and are similar to those required in many countries for licensed handling of "exotic" plant viruses.

CaMV or its DNA may also be useful as a vector to introduce genes into plant protoplasts. The fragility of plant protoplasts combined with the properties of CaMV mentioned above provides adequate safety. Since the group envisaged no risks to the environment from use of the CaMV protoplast system, no special containment was recommended.

Appendix E

TABLE 1

Risk of Illness in Laboratory Workers and of Spread of Viruses to the Environment

Agent	Genome	Severity of Disease in Adult Humans	Risk of Laboratory Infection	Transmission into Community or Environment	Community or Environmental Impact
Lassa Fever Virus	RNA - Negative strand, segmented	4+	H	L - H	H
Variola Virus	DNA - Linear, double stranded	4+	H (If not vaccinated)	L - H	H
*Yersinia pestis (Plague bacterium)		4+	H	L - H	L - H
Yellow Fever Virus (Wild type)	RNA - Positive strand	3+	H (If not vaccinated)	Dependent on presence of vector (H	
*Rickettsia prowazeki (Typhus agent)		3+	H	L	0
Hepatitis B Virus	DNA - Circular, partially single stranded	3+	L to H	L	0 - L
*Vibrio comma (Cholera bacterium)		2+ - 3+	L	L	0 to L
Influenza Virus (Novel epidemic strain)	RNA - Negative strand, segmented	1+ - 2+	L	H	H
Epstein-Barr Virus	DNA - Linear, double stranded	2+	L	L	0
Polio virus (wild)	RNA - Positive strand	0 - 3+	L	L	0 to L
Coxsackie Viruses	RNA - Positive strand	0 - 2+	L	L	0 to L
Herpes simplex virus	DNA - Linear, double stranded	1+	L	L	0
*Shigella sonnei		1+	L	L	0
Vesicular stomatitis virus	RNA - Negative strand	1+ - 2+	H	L	L for humans; H for certain animals.
Adenovirus 2 or 5	DNA - Linear, double stranded	0 - 1+	0 - L	L	0
Adeno-SV40 Hybrid Viruses (Nondefective)	DNA - Linear, double stranded	0 - 1+	0 - L	L	0
Vaccinia virus	DNA - Linear, double stranded	1+	L	L	0
Human Papilloma virus	DNA - Circular, double stranded	1+	L	L	L to 0
Influenza Virus (PR8)	RNA - Negative strand, segmented	0	0	0	0
*Bacteroides sp.		0 - 1+	0	L	0
Adeno-associated virus	DNA - Linear, single stranded	0	L	L	0
BK Virus	DNA - Circular, double stranded	0	L	0	0
Simian Virus 40 (SV40)	DNA - Circular, double stranded	0	L to H (?)	0 to L(?)	0
Sindbis virus (Lab. adapted)	RNA - Negative strand**	0 - 1+	0	Dependent on presence of vector (0-L)	
Semliki Forest Virus	RNA - Negative strand**	0 - 1+	0	Dependent on presence of vector (0-L)	
Reoviruses	RNA - Double stranded, segmented	0	0 to 1+	0	0
Rous sarcoma virus	RNA (Retrovirus)	0	0	0	0
Murine leukemia sarcoma viruses	RNA "	0	0	0	0
Feline leukemia viruses	RNA "	0	0	0	0
Polyoma virus	DNA - Circular, double stranded	0	0	0	0

* Non-viral agents.

** Ed. note: This should read "RNA-Positive strand."

H = High probability; L = Low probability.

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APPENDIX F.—REPORT OF THE VIRUS WORKING GROUP SPONSORED BY THE RECOMBINANT DNA ADVISORY COMMITTEE

On April 6-7, 1978, a working group of American virologists met in a public meeting at the National Institutes of Health to: (1) Review the report of the U.S.-EMBO workshop to assess risks for recombinant DNA expriments involving the genomes of animal, plant, and insect vectors and (2) Translate the conclusions of the U.S.-EMBO report into recommended physical and bilogical containment levels for consideration by the RAC for incorporation into the revised NIH guidelines for recombinant DNA research. The 19 scientists in attendance (see attached roster) had expertise in clinical infectious disease; epidemiology of viral disease; medical and diagnostic virology; the biology of virus infection; biochemical virology; viral immunology; and plant, insect, and veterinary viruses. Six of the participants (Drs. Ginsberg, Choppin, Martin, Rowe, Summers, and Wagner) had attended the U.S.-EMBO workshop.

REVIEW OF THE U.S.-EMBO WORKSHOP REPORT

The working group discussed the U.S.-EMBO workshop report section by section and recommended the following amendments:

(1) Page 10, line 17—delete "then modifies" and insert "is thought to modify."

(2) Page 16, lines 6-8—The working group did not understand the scientific basis behind this short paragraph which singled out viroids from other RNA plant viruses and which would postpone work in this area. Deletion of these three lines was recommended following a discussion about the potential risks attending the cloning of cDNA copies of viroids in *E. coli* K12.

(3) Table 1.

(a) Yellow fever virus—Add "L (if vaccinated)" under Risk of Laboratory Infection and change "(H)" to "(L-H)" under Community or Environmental Impact.

(b) Epstein-Barr Virus—Change "2+" to "0-2+" under Severity of Human Disease.

After reviewing the U.S.-EMBO report the working group unanimously endorsed: (1) The classification of viruses with respect to their ability to cause disease in laboratory workers and their impact on the environment; (2) The analysis and recommendations for cloning viral DNA's in *E. coli* K12; and (3) The analysis and recommendations for the use of viral DNA's as vectors in eukaryotic cells.

IMPLEMENTATION OF THE U.S.-EMBO REPORT

A. Cloning Eukaryotic Viral DNA in *E. coli* K12 Host-Vector Systems.

After considerable discussion, the working group recommended physical and biological containment conditions for experiments involving the cloning of viral DNA in *E. coli* K12 as shown in table 1. The group classified the viral DNA for such experiments in three categories: (1) Virion DNA of DNA viruses; (2) cDNA copies of virion RNA of RNA viruses; and (3) Intracellular forms of viral DNA including integrated genomes, and DNA from productively infected cells. The viral DNA to be cloned was then subclassified into whether it represented the entire viral genome or a purified subgenomic segment thereof. Subgenomic segments were further subdivided on the basis of whether they contained intact transforming genes or not (see table 1). In addition, the working group also assigned physical and biological containment levels for cDNA copies of cellular mRNA's for each class of viral DNA insert (see table 1).

1. In its deliberations, the working group was impressed with the safeguards afforded by a ban on mouth-pipetting for recombinant DNA experiments involving *E. coli* K12 host-vectors. The group felt that the only plausible way *E. coli* K12 could gain entry into laboratory workers was by oral ingestion. The analysis contained in the U.S.-EMBO report was predicated on the remote possibility that *E. coli* K12, containing eukaryotic viral DNA, would be swallowed and the viral DNA insert would be delivered to a tissue in the body which ordinarily would be inaccessible to the virus. A prohibition of mouth pipetting would clearly prevent this sequence of events from even beginning. The working group therefore recommended that no mouth pipetting be allowed at any level of physical containment (including P1) when working with *E. coli* K12.

2. The group was struck by the inherent safety afforded by nonmobilizable plasmids and felt they represented an additional level of containment when used with *E. coli* K12. Accordingly, the working group recommended the use of *E. coli* K12 in conjunction with non-mobilizable plasmid vectors (referred to in table 1 as EK1NM) for several categories of experiments.

3. The virus working group extensively discussed the use of class III (CDC) and "moderately oncogenic" (NCI) viral DNA's in *E. coli* K12 host vector systems. The group concluded that vesicular stomatitis virus (VSV), which is a widely studied negative-strand RNA virus, is classified as a class III agent because of its ability to produce disease in animals; VSV is not an important human pathogen (see table 1 of U.S.-EMBO report). The working group agreed that VSV possessed all of the safety features of other negative-strand RNA viruses (see U.S.-EMBO report) and strongly recommended that cloning of VSV cDNA be permitted because of its importance as a model virus in studies of the molecular biology of virus infection. The working group also considered the 9 viruses classified as moderately oncogenic by NCI (Appendix B) and concluded that the only potential biohazard associated with their use involved the propagation of the viruses themselves during the preparation of reagents/substrates for use in recombinant DNA experiments. None of these agents produces human disease or has been associated with human malignancy. The virus working group endorsed the inclusion of these agents as sources of eukaryotic viral DNA for cloning in *E. coli* K12 and recommended that NCI guidelines be followed for work involving the viruses themselves.

B. Viral DNA Vectors in Eukaryotic Cells.

The working group discussed the physical containment levels appropriate for different eukaryotic viral DNA vectors and prepared a list of four animal viruses and two plant viruses as candidate vectors. In each case the group made recommendations regarding physical containment conditions for productive or non-productive virus-cell interactions. The virus working group, like the participants of the U.S.-EMBO workshop, were concerned about alterations in host range and tissue tropism that might occur following the insertion of foreign viral DNA sequences into eukaryotic viral DNA vectors and were unable to specify containment levels for this type of DNA recombinant. The group agreed that experiments of this type could yield useful information about viral pathogenesis and recommended that each be evaluated by the recombinant DNA Molecule Program Advisory Commitee on a "case-by-case" (CBC) basis. The working group also recognized its inability to identify all possible viral vectors and therefore included a category which encompassed other potential viral DNA vectors whose use could also be evaluated on a "case-by-case" basis.

The virus working group regarded the use of DNA vectors prepared from CaMV and BGMV as posing virtually no biohazard to plants or the ecosystem and recommended a minimum of physical containment. The group extensively discussed the use of baculovirus DNA as vectors, and, despite their certification as registered pesticides, considered the available information about their host range, persistence, and basic biology to be too rudimentary at the present time. A "case-by-case" evaluation was therefore recommended.

The virus working group unanimously endorsed the elimination of the requirement pertaining to the functional anatomy of viral DNA vectors (page 49602, third column, (iii)) since these features are related in no way to the inherent safety of a potential vector. These criteria for candidate vectors were retained in the final recommendations, however, as desirable, although not required features.

It should be noted that the recommendations of the virus working group impact on two other sections of the Revised Guidelines for Recombinant DNA Research.

(1) Section III B 1a(1)(g) can be eliminated because it is now covered in section III B 1b(1).

(2) Section III A should be amended to read "pathogenic organisms in classes 3, 4, and 5 except vesicular stomatitis virus;" the prohibition of moderate risk oncogenic viruses should be deleted.

The recommendations made by the virus working group were based on the best and most current available scientific considerations. The containment levels proposed for cloning eukaryotic

viral DNA inserts in prokaryotic systems pertain *only* to *E. coli* K12 and its derivatives. The working group agreed with the participants of the U.S.-EMBO Workshop that eukaryotic viral genomes cloned in *E. coli* K12 posed less risk in nearly all cases than work with the infectious virus itself.

Recombinant DNA Molecule Program Advisory Committee Working Group on Report of the U.S.-EMBO Workshop to Assess Risks for Recombinant DNA Experiments Involving the Genomes of Animal, Plant and Insect Viruses, National Institutes of Health, Conference Room 9, Building 31C, Bethesda, Md. 20014, April 6-7, 1978, 9 a.m

Ginsberg, Dr. Harold S. (Chairman), Department of Microbiology, Columbia College of Physicians and Surgeons, on Sabbatical at: Rockefeller University, 1230 York Ave., New York, N.Y 10021, 212-360-1977·

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Recommended Containment for Cloning of Viral DNA in E. coli K12 Host-Vector Systems TABLE 1.

Virus Class	Type of Viral DNA Segment to be Cloned: Subgenomic[1] — Non-transforming Segments	Subgenomic[1] — Segments containing an entire transforming gene	Genomic[2] — Non-segmented genome	Genomic[2] — Segmented genome	cDNA from cellular mRNA[3]
DNA	All P1[4]+EK1	All P2+EK1NM[5]			
Non-transforming viruses					
AAV, MVM, Mouse Adeno			P1+EK1		P1+EK1
Other			P1+EK1NM		P1+EK1
Transforming viruses					
Herpes saimiri and H.ateles[6]			P2+EK2 or P3+EK1		
Other			P2+EK1NM		
RNA					
Retroviruses	All P1+EK1	All P2+EK1NM			All P2+EK1NM
Gibbon and woolly monkey			P2+EK2 or P3+EK1		
Other			P2+EK1NM		
Negative strand RNA	P1+EK1		P1+EK1	P1+EK1	P1+EK1
Plus strand RNA	P1+EK1				
Type 1 and 2 Sabin polio, 17D yellow fever vaccine strains			P1+EK1		P1+EK1
Other			P2+EK1NM		P2+EK1NM
Double stranded RNA	P1+EK1			P1+EK1	P1+EK1

1 ≥ 99% pure (i.e., less than 1% of the DNA consists of intact viral genomes), otherwise as for whole genomes.

2 Integrated genomes to be cloned at containment as for shotgun experiments on uninfected cells.

3 ≥ 99% pure, otherwise as for shotgun with uninfected cells.

4 For the purposes of this chart, P1 is defined as including a ban on mouth pipetting.

5 EK1NM means K-12 with non-mobilizable plasmid vectors.

6 These viruses have been classified by NCI as "moderate risk oncogenic viruses," and NCI recommends that the viruses be handled under the equivalent of P3 containment.

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TABLE 2.

Recommended Containment for Recombinant DNA Research Using Eukaryotic Viral Vectors

Vector DNA	Productive Virus-Cell Interactions						Non-Productive Virus-Cell Interactions
	Type of DNA Insert						
	Prokaryotic		Eukaryotic			Eukaryotic Viral	
	Shotgun	Purified	Shotgun		Purified		
			Natural Host	Other			
1. Polyoma							
Intact Genome	P2	P2	P2	P3	P2	CBC*	P2
Deleted Genome	P2	P2	P2	P3	P2	CBC	P2
2. SV40							
Intact Genome	--	--	--	--	--	---	P2
Deleted Genome	P2	P2	P2	P3	P3	CBC	P2
3. Human Ad2+Ad5							
Deleted Genome	P3	P3	P3	P3	P3	CBC	P2
4. Mouse Adenovirus (Strain FL)							
Intact Genome	CBC	CBC	CBC	CBC	CBC	CBC	P2
Deleted Genome	P2	P2	P2	P2	P2	CBC	P2
5. Insect Viruses	CBC	CBC	CBC	CBC	CBC	CBC	--
6. Plant Viruses (CaMV and BGMV)	P1	P1	P1	P1	P1	CBC	--
Plants and Potentially Propagated Plant Parts	Insect-Proof Growth Cabinet or Controlled-Access Insect-Proof Greenhouse.					CBC	--
7. All other potential Viral Vectors	CBC	CBC	CBC	CBC	CBC	CBC	CBC

* CBC - Case by case to be decided by RAC

(1) *Viruses of Eukaryotes.*

(a) *DNA viruses.*

1. *Nontransforming viruses.*

(a) *Adeno-associated viruses, minute virus of mice, and mouse adenovirus strain FL.*—P1 physical containment including no mouth pipetting + an EK1 host-vector shall be used for DNA recombinants produced with the whole genome, subgenomic DNA segments, or cDNA copies of cellular mRNA.*

(b) *Other viruses.*

(i) P1 physical containment including no mouth pipetting + an EK1 host-vector shall be used for DNA recombinants produced with purified subgenamic segments or cDNA copies of cellular mRNA.*

(ii) P1 physical containment including no mouth pipetting + an EK1 host vector, which, in the case of a plasmid, must be non-mobilizable shall be used for DNA recombinants produced with the whole genome.

2. *Transforming viruses.*

(a) *Herpes saimiri and herpes ateles.*

(i) P1 physical containment including no mouth pipetting + an EK1 host vector shall be used for DNA recombinants produced with purified nontransforming subgenomic DNA segments or cDNA copies of cellular mRNA.*

(ii) P2 physical containment + an EK1 host vector which, in the case of a plasmid, must be non-mobilizable, shall be used for DNA recombinants produced with purified subgenomic DNA segments containing an entire transforming gene.

(iii) P3 physical containment + an EK1 host-vector or P2 + EK2 shall be used for DNA recombinants produced with the whole genome.

(b) *Other viruses.*

(i) P1 physical containment including no mouth pipetting + an EK1 host-vector shall be used for DNA recombinants produced with purified non-transforming subgenomic DNA segments or cDNA copies of cellular mRNA.*

(ii) P2 physical containment + an EK1 host-vector which, in the case of a plasmid, must be non-mobilizable, shall be used for DNA recombinants produced with purified subgenomic DNA segments or purified cDNA copies of cellular mRNA.* containing an entire transforming gene.

(b) *RNA viruses.*

1. *Retroviruses.*

(a) *Gibbon ape and woolly monkey viruses.*

(i) P1 physical containment including no mouth pipetting + an EK1 host-vector shall be used for DNA recombinants produced with purified non-transforming subgenomic DNA segments.

(ii) P2 physical containment + an EK1 host-vector which, in the case of a plasmid, must be non-mobilizable, shall be used for DNA recombinants produced with purified subgenomic DNA segments containing an entire transforming gene or cDNA copies of cellular mRNA.*

(iii) P2 physical containment + an EK2 host-vector shall be used for DNA recombinants produced with the whole genome.

(b) *Other viruses*

(i) P1 physical containment including no mouth pipetting + an EK1 host-vector shall be used for DNA recombinants produced with purified non-transforming subgenomic DNA segments.

(ii) P2 physical containment + an EK1 host-vector which, in the case of a plasmid, must be non-mobilizable, shall be used for DNA recombinants produced with purified subgenomic DNA segments containing an entire transforming gene, the whole genome, or cDNA copies of cellular mRNA.*

2. *Negative strand viruses.*—P1 physical containment including no mouth pipetting + an EK1 host-vector shall be used for DNA recombinants produced with the whole genome, subgenomic DNa segments or purified cDNA copies of cellular mRNA.*

3. *Plus-strand RNA viruses.*

(a) *Types 1 and 2 Sabin poliovirus and strain 17D (Theiler) of yellow fever virus.*—P1 physical containment including no mouth pipetting + an EK1 host-vector shall be used for DNA recombinants produced with the whole genome, subgenomic DNA segments or purified cDNA copies of cellular mRNA.*

(b) *Other viruses*

(i) P1 physical containment including no mouth pipetting + an EK1 host-vector shall be used for DNA recombinants produced with purified subgenomic DNA segments.

(ii) P2 physical containment + an EK1 host-vector which, in the cse of a plasmid, must be nonmobilizable, shall be used for DNA recombinants produced with the whole genome or purified cDNA copies of cellular mRNA.*

4. *Double-stranded segmented RNA viruses.*—P1 physical containment including no mouth pipetting + an EK1 host vector shall be used for DNA recombinants produced with mixtures of subgenomic segments, a specific subgenomic segment, or purified cDNA copies of cellular mRNA.*

5. *Viroids.*—P1 physical containment including no mouth pipetting + an EK1 host-vector shall be used for DNA recombinants produced with the whole genome, subgenomic DNA segments or cDNA copies of cellular mRNA.*

(c) *Intracellular viral DNA.*—Physical and biological containment specified for shotgun experiments involving uninfected eukaryotic cellular DNA [sec. B.1.a.(1)] shall be used for DNA recombinants produced with integrated viral DNA or viral genomes present in infected cells.

3. *Experiments with Eukaryotic host-vectors.*

a. *Vertebrate host-vector systems.*—Because this work will be done almost exclusively in tissue culture cells, which have no capacity for propagation outside the laboratory, the primary focus for containment is the vector; it should be pointed out that risk of laboratory acquired infection as a consequence of tissue culture manipulations is very low. Given good microbiological practices, the most likely mode of escape of recombinant DNAs from a physically contained laboratory is carriage by an infected human; thus the vector with an inserted DNA segment should have little or no ability to replicate or spread in humans. Further, a recombinant virus should not inadvertently pose a threat to any species.

For use as a vector in a vertebrate host cell system, an animal viral DNA molecule should display the following properties:

(a) It should not consist of the whole genome of any agent that is infectious for humans or that replicates to a significant extent in human cells in tissue culture. If the recombinant molecule is used to transform non-permissive cells (i.e. cells which do not produce infectious virus particles), this is not a requirement.

(b) It should be derived from a virus whose epidemiological behavior and host range are well understood.

(c) In permissive cells, it should be defective when carrying an inserted DNA segment; (i.e. propagation of the recombinant DNA as a virus must be dependent upon the presence of a complementing helper genome). In almost all cases this condition would be achieved automatically by the manipulations used to construct and propagate the recombinants. In addition, the amount of DNA encapsidated in the particles of most animal viruses is defined within fairly close limits. The insertion of sizeable foreign DNA sequences, therefore, generally demands a compensatory deletion of viral sequences. It may be possible to introduce very short insertions (50–100 base pairs) without rendering the viral vector defective. In such a situation, the requirement that the viral vector be defective is not necessary except in those cases in which the inserted DNA encodes a biologically active polypeptide.

It is desired but not required that the functional anatomy of the vector be known—that is, there should be a

*The cDNA copy of cellular mRNA must be 99 percent pure; otherwise, physical and biological containment specified for shotgun experiments involving uninfected eukaryotic cellular DNA [sec. B.1.a.(1)] shall be used.

clear idea of the location within the molecule of:

(a) The sites at which DNA synthesis originates and terminates.

(b) The sites that are cleaved by restriction endonucleases.

(c) The template regions for the major gene products.

If possible the helper virus genome should:

(i) Be integrated into the genome of a stable line of host cells (a situation that would effectively limit the growth of the vector recombinant to such cell lines), or

(ii) Consist of a defective genome, or an appropriate conditional lethal mutant virus, making vector and helper dependent upon each other for propagation.

However, neither of these stipulations is a requirement.

(1) *Polyoma virus.*

(a) *Productive virus-cell interactions.*

1. Defective or intact polyoma virus genomes, with appropriate helper, if necessary, can be used in P2 conditions to *propagate* DNA sequences from:

(*a*) Bacteria of class 1 or class 2 (see appendix B), or their phages or plasmids, except for species of bacteria that produce potent polypeptide toxins.

(*b*) From mice.

(*c*) From other eukaryotic organisms that do not produce potent polypeptide toxins, provided the DNA segment is purified.

2. Defective or intact virus genomes with appropriate helper, if necessary, can be used in P3 conditions for shotgun experiments to *propagate* DNA sequences from eukaryotic organisms, provided the DNA is obtained from uninfected cells such as embryonic or tissue culture cells.

3. Experiments involving the use of defective polyoma virus genomes to *propagate* DNA sequences from eukaryotic viruses will be evaluated by the Recombinant DNA Molecule Program Advisory Committee on a case-by-case basis and will be conducted under physical containment conditions recommended by that committee.

(b) *Non-productive virus-cell interactions.*—Defective or intact polyoma virus genomes can be used as vectors in P2 conditions to *transform* nonpermissive cells in culture.

(2) *Simian virus 40.*

(a) *Productive virus-cell interactions.*

1. SV40 DNA, rendered unconditionally defective by a deletion in an essential gene, with appropriate helper, if necessary, can be used in P2 conditions to *propagate* DNA sequences from:

(*a*) Bacteria of class 1 or class 2 (see appendix B), or their phages or plasmids, except for species of bacteria that produce potent polypeptide toxins.

(*b*) Uninfected African green monkey kidney cells.

2. SV40 DNA, rendered unconditionally defective by a deletion in an essential gene, with an appropriate helper, if necessary, can be used in P3 conditions to *propagate* DNA sequences from eukaryotic organisms (shotgun experiments or purified DNA) provided the DNA is obtained from uninfected cells such as embryonic or tissue culture cells.

3. Experiments involving the use of defective SV40 genomes to *propagate* DNA sequences from eukaryotic viruses will be evaluated by the Recombinant DNA Molecular Program Advisory Committee on a case-by-case basis and will be conducted under physical containment conditions recommended by that committee.

(b) *Non-productive virus-cell interactions.*—Defective or intact SV40 genomes can be used as vectors in P2 conditions to *transform* nonpermissive cells in culture.

(3) *Human adenoviruses 2 and 5.*

(a) *Productive virus-cell interactions.*

1. Human adenoviruses 2 and 5, rendered unconditionally defective by deletion of at least 2 capsid genes, with appropriate helper(s), if necessary, can be used in P3 conditions to *propagate* DNA sequences from:

(*a*) Bacteria of class 1 or class 2 (see appendix B) or their phages or plasmids except for species of bacteria that produce potent polypeptide toxins.

(*b*) Eukaryotic organisms (shotgun experiments or purified DNA) provided the DNA is obtained from uninfected cells such as embryonic or tissue culture cells.

2. Experiments involving the use of unconditionally defective human Ad 2 and 5 genomes to *propagate* DNA sequences from eukaryotic viruses will be evaluated by the Recombinant DNA Molecule Program Advisory Committee on a case-by-case basis and will be conducted under physical containment conditions recommended by that committee.

(b) *Non-productive virus-cell interactions.*—Defective or intact human Ad 2 and 5 genomes can be used as vectors in P2 conditions to *transform* non-permissive cells in culture.

(4) *Murine adenovirus strain FL.*

(a) *Productive virus-cell interactions.*

1. Unconditionally defective murine adenovirus strain, FL genomes, with appropriate helper, if necessary, can be used in P2 conditions to *propagate* DNA sequences from:

(*a*) Bacteria of class 1 or class 2 (see appendix B) or their phages or plasmids except for species of bacteria potent polypeptide toxins.

(*b*) Eukaryotic organisms (shotgun experiments or purified DNA) provided the DNA is obtained from uninfected cells such as embryonic or tissue culture cells.

2. Experiments involving the use of intact murine adenovirus strain FL genomes to *propagate* DNA sequences from prokaryotic or eukaryotic organisms will be evaluated by the Recombinant DNA Molecule Program Advisory Committee on a case-by-case basis and will be conducted under physical containment conditions recommended by that committee.

3. Experiments involving the use of unconditionally defective murine adenovirus strain FL genomes to *propagate* DNA sequences from eukaryotic viruses will be evaluated by the Recombinant DNA Molecule Program Advisory Committee on a case-by-case basis and will be conducted under physical containment conditions recommended by that committee.

(b) *Non-productive virus-cell interactions.*—Defective or intact murine adenovirus strain FL genomes can be used as vectors in P2 conditions to *transform* non-permissive cells in culture.

(5) *All other potential viral vectors.*

(a) Experiments involving the use of viral DNA vectors consisting of 25 percent or less of the viral genome shall be used:

1. In P2 conditions to *transform* nonpermissive cells in culture.

2. Under physical containment conditions to be determined by the Recombinant DNA Molecule Program Advisory Committee to *propagate* DNA sequences from prokaryotic or eukaryotic organisms.

(b) Experiments involving the use of other intact or defective virus genomes to *propagate* DNA sequences from prokaryotic or eukaryotic organisms (and viruses) or as vectors to *transform* non-permissive cells will be evaluated by the Recombinant DNA Molecule Program Advisory Committee on a case-by-case basis and will be conducted under physical containment conditions recommended by that committee.

The Recombinant DNA Molecule Program Advisory Committee will also review all experiments involving the use of virus vectors in animals and the physical containment conditions appropriate for such studies.

b. *Invertebrate host-vector systems in which insect viruses are used to propagate other DNA segments.*—As soon as information concerning the nature of the host range, infectivity, persistence and integration in vertebrate and invertebrate cells becomes available, experiments involving the use of baculo-viruses to propagate

DNA sequences will be evaluated by the Recombinant DNA Molecule Program Advisory Committee on a case-by-case basis and will be conducted under physical containment conditions recommended by the committee. Experiments should be done in established invertebrate cell lines and should follow, where appropriate, criteria recommended for vertebrate viral DNA vectors [Sec. 3.a.(a-c)].

c. *Plant host-vector systems.*—The DNA plant viruses which could currently serve as vectors for cloning genes in plants and plant cell protoplasts are Cauliflower Mosaic Virus (CaMV) and its close relatives, which have relaxed circular double stranded DNA genomes with a molecular weight of 4.5×10^6, and Bean Golden Mosaic Virus (BGMV) and related viruses with small ($<10^6$ daltons) singled-stranded DNA genomes. These viruses are not known to integrate into host chromosomes, or to incorporate cellular genes into their genomes. CaMV is spread in nature by aphids, in which it survives for a few hours. Spontaneous mutants of CaMV that are not transmitted by aphids arise frequently; these mutants fail to make a transmission factor essential for aphid transmission. BGMV is spread in nature by whiteflies, in which it survives for several days to three weeks; certain other single-stranded DNA plant viruses are transmitted by leafhoppers, in which the viruses persist for days or weeks. Single-stranded DNA plant viruses are thought not to replicate in their insect vector.

The DNA plant viruses have narrow host ranges and are relatively difficult to transmit mechanically to plants. For this reason, they are most unlikely to be accidentally transmitted from spillage of purified preparations of the virus.

When these viruses are used as vectors with intact plants, the plants should be grown in either a limited access greenhouse or plant growth cabinet which is insect-proof, preferably with positive air pressure, and in which an insect fumigation regime is maintained. Soil, plant pots and unwanted infected plant materials should be removed from the greenhouse or cabinet in sealed insect proof containers and sterilized. It is not necessary to sterilize run-off water from the infected plants as this is not a plausible route for secondary infection. Infected plant materials to be used for further research, which have to be removed from the greenhouse or cabinet, should be maintained under insect proof conditions. These measures provide an entirely adequate degree of containment and are similar to those required in many countries for licensed handling of "exotic" plant viruses.

The viruses or their DNA may also be useful as a vector to introduce genes into plant protoplasts. The fragility of plant protoplasts combined with the properties of the viruses mentioned above provide adequate safety. Since no risk to the environment from the use of the DNA plant virus/protoplast system is envisaged, no special containment is recommended.

Experiments involving the use of plant virus genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by the Recombinant DNA Molecule Program Advisory Committee on a case-by-case basis and will be conducted under containment conditions recommended by that committee.

Appendix G.—Report of a Workshop on Risk Assessment of Agricultural Pathogens

Conducted by: Recombinant DNA Molecule Program Advisory Committee.

Sponsored by: U.S. Department of Agriculture, National Science Foundation, and National Institutes of Health, March 20-21, 1978.

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Introduction.
Hazard classification of plant pathogens.
Table 1.—Survival data of plant protoplasts.
Table 2.—Available evidence for exchange among bacterial plant pathogens and symbionts.
References.
Participants.
Liaison representatives.

Introduction

An assessment of risk involved in recombinant DNA research on plant and insect pathogens necessarily entails consideration of different concerns than those applied to risk assessment of research with pathogens of man and animals. This fundamental difference provided the basis on which the recommendations of our committee were formulated.

There was a consensus amongst the committee members that working with plant pathogens and baculoviruses in recombinant DNA studies presents no more hazard than that which exists in current laboratory studies with the pathogens themselves. It is significant that to the best of our knowledge there have been no cases recorded in which laboratory studies with cultures of plant pathogens have resulted in illness in man or animals exposed to these organisms. Neither is there a documented case in which laboratory studies with cultures have resulted in an escape resulting in an outbreak of disease in plants growing under natural conditions.

We believe that any potential risk that might arise from studies involving plant pathogens is now adequately covered by existing federal and state quarantine regulations. These have evolved to enable government to deal with the practicalities of plant disease control (Gram, E., 1960, Chapter 9, pp. 314-356 in "Plant Pathology, An Advanced Treatise", J. G. Horsfall and A. E. Dimond, eds., vol. 3, Acad. Press., New York). They take precedence over any other regulations since they determine whether or not an investigator has access to a particular pathogen.

With plants, resistance to disease is the rule, susceptibility is the exception. Thus, the common occurrence of resistance poses an important barrier to the successful establishment of potential pathogens. There is no pathogen that is highly virulent on all plant species. Rather, the majority of pathogens are restricted to a relatively small number of host plants and within these specific susceptible hosts varietal variation in resistance is usually present. Breeding for disease resistance has provided a means for effecting relatively rapid changes in varieties. In the United States today, approximately 95 percent of the acreage in crops of economic importance is planted to varieties that carry resistance to one or more major diseases. This is one of the key factors in the productivity of American agriculture. Even when a particularly destructive new race or strain of a plant pathogen arises, it is possible to change the available varieties and to reduce or minimize the threat within the space of two or three years. This was the case when a virulent strain of the pathogen that causes *Helminthosporium* leaf blight spread throughout the corn growing area of the United States in 1970 and it was necessary to discontinue the growing of those hybrids that carried the factor for male sterility which also conferred susceptibility to the disease (Ullstrup, A. J., 1972, Ann. Rev. Phytopath. 10:37-50).

Virulent plant pathogens commonly are contained in nature because for their very survival and dissemination within a region, or for an epidemic to develop, they require specific environmental factors (Colhoun, J., 1973, Ann. Rev. Phytopath. 11:343-364). For example, bacterial wilt caused by *Pseudomonas solanacearum* rarely occurs north of the Mason-Dixon line. The causal bacterium is sensitive to low temperatures and has a relatively high optimum temperature for growth. Even when introduced inadvertently into Northern states it does not overwinter in the soil or affect susceptible crops the following year (Kelman, A., 1953, N. Carolina Agr. Exp. Sta. Bull. 99, 194p).

In considering hypothetical risks of recombinant DNA research on plant pathogenic organisms our committee considered the following questions:

1. Will the introduction of recombinant DNA from a specific plant pathogen in *E. coli* K12 lead to the development of strains with enhanced virulence to man or animals?

2. Will strains of *E. coli* be converted into plant pathogens?

3. Will the use of plant pathogens as HV systems result in hazards to plants?

With the relatively minor reservations documented below, the committee agreed that the answer to all three questions was "No". First we would point out that there is no evidence that genetic information transferred from plant pathogenic organisms would enhance the capability of strains of *E. coli* to harm man, animals or plants. There are a very small number of possible exceptions to this generalization. These include a few species of bacteria which have been found in association with plants or which have been described as weak or minor plant pathogens and which may be closely related to forms causing disease in man. Also, plant pathogens such as the ergot fungus, and certain molds that produce aflatoxins on stored plant products are likely to be banned on the grounds that they produce potent, albeit non-polypeptide, toxins.

Provided that the use of plant pathogens as HV systems is not undertaken with the objective of deliberately creating forms with increased virulence and host range beyond that which occurs by natural genetic exchange (these are expressly prohibited by the guidelines) we see no hazard from such systems to plants.

1. Without dissent, the committee agreed on a classification of plant pathogens on the basis of hazard to agriculture.

We have placed all plant pathogens into a single class with two subgroups. This reflects our opinion that recombinant DNA research with plant pathogens has a negligible risk. The two subgroups take account of existing federal and state quarantine regulations. We propose that this classification be appendix C in the guidelines.

HAZARD CLASSIFICATION OF PLANT PATHOGENS

Class 1A—Plant pathogens not in class 1B.

Class 1B—All organisms that are subject to quarantine restrictions for any of the following reasons:

(i) Plant pathogens not known to occur in the United States.

(ii) Plant pathogens that are not widely distributed throughout the ecological range of their hosts.

(iii) Plant pathogens subject to federal or state eradication or suppression programs.

All plant pathogens require state and federal (USDA[1]) permits for shipment across state lines.

2. *Specific recommendations.* The lack of an accepted definition of exchangers caused us some difficulty in our discussions. A majority of the committee favor a liberal definition that would exclude all gram negative bacteria from the guidelines. However, since this decision has still to be made by the RAC and Dr. Fredrickson some of our recommendations had to reflect two alternatives: (i) On the basis that most gram negative forms would be excluded we present rationale for including gram negative plant pathogens in this exclusion; (ii) on the basis that prokaryotic exchangers might be defined in a less liberal fashion, or that there might be a long lag period before plant pathologists can present evidence satisfying whatever exchange criteria are established, we present rationale for adopting minimal containment levels for all phytopathogenic bacteria namely P1+EK2 or P2+EK1.

III B 1a. Shotgun experiments using the *E. coli* K12 host-vector systems.

(2) Prokaryotic DNA recombinants. (p. 49602 FR 42 No. 187, Sept. 27, 1977). We propose that the minimum containment levels adopted for other bacteria be applicable to phytopathogenic bacteria, namely P1+EK2 or P2+EK1.

(i) Modify line 7 to read; "biochemical, genetic, and/or pathogenic properties.

(ii) Delete the words "and plant pathogens" from line 5 of the second paragraph.

Rationale: Plant pathogenic bacteria include diverse organisms principally in the genera *Agrobacterium, Corynebacterium, Erwinia, Pseudo- monas,* and *Xanthomonas.* A number of the plant pathogenic species are soil-inhabitants and are widely distributed throughout the United States. In general they cause economic loss only when environmental conditions are favorable and available control practices are not used. A number of bacteria that cause foliage diseases can exist as epiphytes on a variety of non-host plants as well as on their susceptible hosts. Other bacteria such as the pathogen that causes halo blight of beans are seed-transmitted, do not survive in soil for long periods of time and can be controlled by the use of pathogen-free seed. The mechanisms by which plant pathogenic bacteria produce disease in plants may involve enzymes which attack substrates in plants such as pectic compounds. Such enzymes would not be harmful to man or animals. Similarly certain growth promoting compounds and compounds that interfere with specific physiological functions in plants, e.g. polysaccharides that block movement of water, are not known to cause injury to man.

Certain bacteria which are not known to cause disease in plants but which are commonly present as epiphytes on leaves (Leben, C., 1965 Ann. Rev. Phytopath. 3:209-230) have been associated with diseases of man, i.e. *Erwinia herbicola* also designated as *Enterobacter agglomerans* (Starr, M. P. and Chatterjee, A. R., 1972, Ann. Rev. Microbiol. 26:389-426). However, there is a diversity of strains that have been obtained from plants and there is no conclusive evidence that the types widespread in plants are the same strains associated with certain infections in man.

Similarly it has been reported that a bacterium similar to a pathogen of onions (*Pseudomonas cepacia*) has been associated with a disease of man (Ederer, G. M. & Matsen, J. M., 1972, J. Infect. Dis. 125:613-618; Snell, J. J. S., Hill, L. R., LaPage, S. P. & Curtis, M. A., 1972, Internat. Symp. Systemat. may not have useable vectors.

III.B. 3c. Plant host-vector systems. (p. 49603).

(i) Delete second paragraph. "Whole plants or plant * * * at this time".

Rationale: This paragraph is confusing. Its intent was to define practical size or scale limits to physical containment rather than limits to biological containment. The committee concluded that the discussion of physical containment in the preceding paragraph makes this redundant.

(ii) Delete last sentence of fourth paragraph "However, if the source of the DNA is itself pathogenic * * * shall be carried out under P3 conditions" and substitute: "If the vector is an unmodified virus the experiments shall also be carried out under P2 conditions".

Rationale: This committee has reassessed the risk to man, animals, and plants from plant pathogenic agents. In this context we are concerned principally with the risk of DNA from plant pathogens to plants. This paragraph now reflects our lowered assessment of these risks.

(iii) Delete paragraph five. Experiments on * * * are not met.

Rationale: As for (ii).

(iv) Modify final paragraphs to read "* * * permit a decrease of one step in the physical containment to P1."

Rationale: The survival of plant protoplasts (see table 1) and undifferentiated cultured plant cells outside their laboratory environment is zero because of their extremely exacting growth requirements and fragility.

[1]Address to obtain application to import or move a plant pest or pathogen: Plant Importation and Technical Support Staff, Plant Protection and Quarantine Programs, Animal and Plant Health Inspection Service, USDA, Federal Center Building, Hyattsville, Md. 20782.

II.D. a. HV-1 (p. 49600).

This committee proposes that the Recombinant Advisory Committee consider allowing the construction of modified HV-1 systems with conjugation proficient plasmids in addition to other recombinant molecules of prokaryotic origin under one step higher physical containment providing that all the DNA segments in the cell are derived from organisms which exchange DNA by natural physio-Lower Eukaryotes (p. 9601)(e)2. Insert the words—

The remainder of the species in this class, including plant pathogenic or symbiotic fungi that do not produce potent toxins: P2+EK1 or P1+EK2.

Rationale: There is no demonstrable risk either to man or plants from cloning such DNA in *E. coli*. Also the present wording which refers to disease causing microorganisms could be interpreted to call for an unreasonably high containment level for these plant pathogens.

(f) Plants. (p. 49601). Delete the words "carries a known pathogenic micro-organism or"

Rationale: The risk to man or plants from DNA of a plant pathogen is not comparable to the risk of cloning DNA which codes for a potent polypeptide toxin. We have covered this risk elsewhere.

II.D. Biological containment—Host vector systems. a.2. Other prokaryotes (p. 49600).

We endorse the La Jolla Working Group Draft: (Insert III-3). "Experiments that are exempt from these guidelines". In the event that sections (iii) and (iv) are not adopted we propose the following:

Self-cloning of bacterial plant pathogens and symbionts:

(i) The use of an indigenous plasmid or bacteriophage shall be exempt from the guidelines.

(ii) The use of a foreign vector (a non-indigenous plasmid or bacteriophage) from an organism which exchanges DNA by natural physiological processes shall require P2 containment.

Rationale: Many self-cloning experiments with agriculturally significant gram-negative bacteria could be more readily and safely carried out by using well characterized *E. coli* plasmid vectors. Some plants pathogens and symbionts Bacteriol., 22:-138). *Pseudomonas aeruginosa*, a pathogen of man, conversely, Has been reported to cause a leaf-spot of tobacco but is considered a minor and inconsequential pathogen of plants (Cho, J. J., Schroth, M., Mason, M N, Komino, S. D. and Green, S. K., 1975 Phytopath. 65:425-431). These three bacteria should be governed by regulations applicable to human pathogens.

In our opinion it is a mistake to equate prokaryotic plant pathogens with Class 2 human pathogens as is done in the revised guidelines. The minimum containment level for those that have been extensively characterized as to pathogenic and other properties should be consistent with that adopted for other prokaryotes namely, P1+EK2 or P2+EK1.

III B1b(1)(d) Viruses of plants (p. 49602). Change to P2+Ek1 or P1+Ek2.

Rationale: Because of their fastidious modes of transmission and restrictive host ranges, DNA plant viruses were considered to present a minimal risk to animals or agriculture when used in shotgun experiments with the *E. coli* K-12 host vector systems.

III B 3b Pesticide baculoviruses (p. 49603).

(1) Remove sentences "Two viruses are presently registered * * * tussock moth".

Rationale: Footnote No. 7 in the September, 1977, draft describes the baculovirus pesticides that have been registered to date. The second sentence of the September, 1977, draft is therefore repetitive. Also, it should be made clear that any baculovirus that is registered by the EPA may be used as a vector since EPA registration is an ongoing process and other baculoviruses may eventually be registered which could be more useful for vector work.

(2) Remove the sentence "However, much still needs to be learned" and rewrite the final sentence of first paragraph to read "However, information is needed on the nature of the host range specificity, particularly the infectivity and persistence of the viral DNA in invertebrate and vertebrate cell cultures."

Rationale: The original sentence, "However much still needs to be learned," introduces ambiguity. The background information that was agreed to be essential in 1977 was information on the host specificity, particularly infectivity of viral DNA in vertebrate cell cultures.

(3) Substitute for the last paragraph in this section: When such background information is available, and if it confirms the narrow host range specificity, a baculovirus vector may be used for cloning DNA segments derived from the host insect, from another Environmental Protection Agency registered baculovirus, from an EK1 bacterium of from DNAs cloned in an EK1 bacterium (with the exception of any cloned DNA derived from an animal virus other than an EPA registered baculovirus), using P2 physical containment. Cloning of other classes of DNA is not envisioned for the exploratory phases of this work, but may be considered on a case-by-case basis in the future.

Rationale: The term "EK1 bacterium" was originally meant to include any DNA cloned in an EK1 bacterium, not simply *E. coli* K-12 DNA. Of particular interest in this category are Lepidopteran genes already cloned in *E. coli* K-12 such as the *B. mori* silk gene and the chorion genes of *A. polyphemus*. Also, it would be of interest to extract baculovirus DNA cloned in EK1 with a plasmid vector and test infectivity and/or effects in insect cell cultures. Such experimentation is also relevant to safety assessment of EK1 hosts.

Rationale: The revised guidelines explicitly prohibit conjugative plasmids and generalized transducing phages in EK-1 and HV-1 Systems. It is implicit that these elements shall not be introduced subsequent to cloning. This precludes host range tests unless they are carried out under more stringent physical containment. It also prevents plasmid promoted mobilization to introduce recombinant molecules back to the *original* DNA source organism or its mutants. Plant pathogens and Rhizobia are often non-transformable. This will effectively preclude complementation tests for traits not expressed in the cloning host such as plant pathogenicity in EK hosts. In effect, this creates a dilemma. In the proposed revisions of the drafted guidelines self-cloning in plasmids will be excluded even though the recombinant DNA can be mobilized out of the host strain into other prokaryotes. On the other hand, DNA cloned from the donor prokaryotes into a different recipient prokaryote cannot be mobilized by a conjugative plasmid back into the original donor, despite the fact that it is receiving its own DNA by this procedure. If the "exchanger" list is based on plasmid exchange these arguments are irrelevant.

We have listed in table 2 our suggestions for prokaryotic exchangers that are either plant pathogens or symbionts (Rhizobium) together with evidence for such exchange.

TABLE 1

SURVIVAL DATA OF PLANT PROTOPLASTS

(S-T. Liu, S. M. Fernandez, N. Schwinghamer, C. I. Kado,
Department of Plant Pathology, University of California
Davis, CA 95616 - March 16, 1978)

Source	Growth Conditions: Medium	Supplemented phyto-hormones	Temperature (° C)	Cell density (/ml)	Cell wall formation	Period of survival (days)	Fraction surviving (%)
(Vigna sinensis)							
Cowpea	G/D[1]	yes	25	$>10^6$	yes	10	10
Cowpea	G/D	no	25	$>10^6$	no	0	0
Cowpea	0.45 M Mannitol 1% agar (sealed)	no	25	$>10^6$	no[2]	0	0
Cowpea	0.45 M Mannitol 1% agar (unsealed)	no	25	$>10^6$	no[3]	0	0
Cowpea	2% water agar	no	25	$>10^6$	no (lysis)	0	0
Cowpea	sterile soil[4]	no	25	$>10^6$	no (lysis)	0	0
Cowpea	unsterile lab bench top	no	25	$>10^6$	no[5]	0	0
(Nicotiana tabacum)							
Tobacco	S/T-III[6]	yes	22	$>10^5$	yes	>21	63[6]
Tobacco	S/T-III	no	22	$>10^5$	no	0	0
(Vinca rosea)							
Periwinkle	M/S[7]	yes	22	$>10^5$	yes	6	1
Periwinkle	M/S	no	22	$>10^5$	no	0	0
(Brassica rapa)							
Turnip	B5[8]	yes	28	10^3-10^4/cm^2	yes[9]	0	0
Turnip	B5 (without sugars)	yes	28	10^3-10^4/cm^2	yes[9]	0	0
Turnip	M/S	yes	28	10^3-10^4/cm^2	yes[9]	0	0
Turnip	G/D	yes	28	10^3-10^4/cm^2	yes[9]	0	0
Turnip	B/W[10]	yes	28	10^3-10^4/cm^2	yes[9]	8	0

[1]Greshoff, P. and C. Doy (1972) Planta 107, 161-170
[2]Dried completely in 12 hours
[3]Dried completely in 3 hours
[4]UC potting mix
[5]Dried in 5-30 min.
[6]Shepard, J.F. and R.E. Totten (1975) Plant Physiol. 55, 689-694
[7]Murashige, T. and F. Skoog (1962) Physiol. Plantarum 15, 473-497
[8]Gamborg, O.L., R.A. Miller, and K. Ojima (1968) Exptl. Cell. Res. 50, 151-158
[9]Protoplast first allowed to regenerate cell walls before plating on media indicated
[10]Braun, A.C. and H.N. Wood (1962) Proc. Natl. Acad. Sci. U.S.A. 43, 1776-1782

TABLE 2

Available evidence for exchange among bacterial plant pathogens

and symbionts (prepared by C. I. Kado)

			plasmid exchange	chromosomal exchange
Agrobacterium	x	Erwinia	-	-
	x	Pseudomonas	1	-
	x	Xanthomonas	23	-
	x	Rhizobium	12,20	29
	x	E. coli	15,10,17,27	-
Erwinia	x	Pseudomonas	18,11	27
	x	Xanthomonas	23	-
	x	Rhizobium	-	-
	x	E. coli	5,7,8,14,21,27	19,9,13
Pseudomonas	x	Xanthomonas	23	-
	x	Rhizobium	15,6,28	3
	x	E. coli	15,11,26,22	-
Xanthomonas	x	Rhizobium	-	-
	x	E. coli	23	-
Rhizobium	x	E. coli	6,28,16,2,4,25	24

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RECOMBINANT DNA MOLECULE PROGRAM ADVISORY COMMITTEE, WORKSHOP ON RISK ASSESSMENT OF AGRICULTURE PATHOGENS

National Science Foundation, Conference Room 338, 1800 G Street NW., Washington, D.C. 20550, March 20-21, 1978, 9 a.m.

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