seeking comment, particularly scientific justification, for changes in the current regulation.

Persons desiring to make an oral presentation at this hearing or who desire to submit a written statement must file a written notice of participation by February 29, 1980, with the Hearing Clerk (HFA–305), Food and Drug Administration, Rm. 4–65, 5600 Fishers Lane, Rockville, MD 20857. The notice of participation should be identified with the Docket No. 80N–0025 and contain the following information:

1. Name, address, and telephone number of person desiring to make a presentation and/or to submit written comments;

2. Business or organizational affiliation, if any;

3. Topic(s) of presentation; and

4. Number of minutes required for an oral presentation (maximum of 15 minutes, unless more time can be justified).

A schedule of presentations for the hearing will be mailed to each person who files a notice of participation and will also be available from the Hearing Clerk. Time permitting, persons not formally scheduled to make a presentation may be allowed to make a presentation at the discretion of the presiding officer. Individuals and organizations with common interests are urged to consolidate their presentations. Formal written statements or extensions of remarks (preferably four copies) may be presented to the presiding officer on the day of the hearing for inclusion in the hearing record of this proceeding.

FDA has established a pilot program for financial assistance to participants in certain agency proceedings, including hearings under Part 15. This program is described in regulations that were published in the **Federal Register** of October 12, 1979 (44 FR 59174) and that became effective on October 25, 1979 (44 FR 72585; December 14, 1979). Subject to the availability of funds and other factors, FDA may reimburse participants meeting the criteria set forth in these regulations for certain costs of participating in this proceeding. For more information regarding the reimbursement program, contact Ron Wylie, Office of Consumer Affairs (HF–70), Food and Drug Administration, Department of Health, Education, and Welfare, 5600 Fishers Lane, Rockville, MD 20857, 301–443–2932. Although reimbursement may be made available for hearings, such as this, under Part 15, the program's priority will be given to funding participation in formal evidentiary public hearings under Part 12 or public boards of inquiry under Part 13 of FDA's regulations (21 CFR Parts 12 or 13).

Applications for reimbursement must be filed by February 25, 1980, in accordance with § 10.210 (44 FR 59186; October 12, 1979).

Dated: January 23, 1980.

Jere E. Goyan,

Commissioner of Food and Drugs.

[FR Doc. 80–2829 Filed 1–28–80; 8:45 am]

BILLING CODE 4110–03–M

federal register

Tuesday
January 29, 1980

Part IV

Department of Labor

Occupational Safety and Health Administration

Servicing Multi-Piece Rim Wheels; Procedures

DEPARTMENT OF LABOR

Occupational Safety and Health Administration

29 CFR Part 1910

Servicing Multi-Piece Rim Wheels

AGENCY: Occupational Safety and Health Administration, U.S. Department of Labor.

ACTION: Final standard.

SUMMARY: By this final standard the Occupational Safety and Health Administration (OSHA) establishes procedures for the servicing of multi-piece rim wheels fitted on vehicles used on and off highways. Multi-piece rim wheels consist of two or more detachable rim components, one of which is a side or locking ring designed to hold the tire on the rim base when the tire is inflated. These wheels are used on motor vehicles, such as trucks, trailers, buses and motor homes, for either on-highway or off-highway usage. The major hazard in servicing multi-piece rim wheels is the possibility of an employee being struck by a wheel component which has been thrown from an inflated wheel during an unintended explosive separation. This standard includes requirements for training of all tire servicing employees, establishment of a safe practice procedure for servicing multi-piece rim wheels, use of restraining devices and criteria for interchangeability of rim components.

EFFECTIVE DATE: This standard will become effective April 28, 1980.

FOR FURTHER INFORMATION CONTACT: William Simms, Occupational Safety and Health Administration, Room N-3106, U.S. Department of Labor, Washington, D.C. 20210, Telephone: (202) 523-8126.

SUPPLEMENTARY INFORMATION: For additional copies of this regulation contact: OSHA Office of Publications, U.S. Department of Labor, Room S-1212, Washington, D.C. 20210, Telephone: 202-523-8677.

A. Background

1. Multi-piece Rims

Multi-piece rims are used in conjunction with tube-type tires, most frequently on trucks, tractors, buses, trailers, campers and off-highway type vehicles. Multi-piece rims consist of two or more components which, when assembled and the tire is inflated, are held together by the force of the air pressure in the tire. Multi-piece rims may consist of up to five or six components on large wheels for off-the-road vehicles.

A multi-piece rim consists of a rim base, the largest part of the metal structure supporting the tire, and one or more detachable side rings serving as a flange to keep the inflated tire on the rim base. The rim base, side ring, lock rings, and tire are collectively referred to as a "wheel."

For multi-piece rims, the rim base and the side or locking rings are the primary components which support the tire's bead. This is referred to as a split side ring in two piece assemblies and a solid side ring and split lock ring in three piece assemblies. In the case of two piece assemblies, the circumferentially continuous outer small component is termed a side ring. (See Society of Automotive Engineers, SAE J393, which defines rim terminology.)

There are basically four multi-piece wheel designs. In the first design (exemplified by Goodyear's "KW" type rim) the rim base is split radially and the side ring is circumferentially continuous. In the second design (exemplified by Firestone's, Kelsey's and Budd's "RH5°" and "KL" rims) both the rim base and the side ring are circumferentially continuous. The third type rim (exemplified by Goodyear's "LW" type rim) is a two piece assembly composed of a demountable rim base and a split side ring. The fourth design in the larger sizes (exemplified by Firestone's "Commander 5°" rim) is a three piece assembly composed of rim base, a side and a lock ring.

2. History of the Regulation

OSHA concern for developing a standard to protect employees engaged in servicing multi-piece rim wheels was initiated by an internal report of "Hazards Not Covered by a Standard" from OSHA field personnel in the Louisville, Kentucky office. This was followed by a similar report from OSHA field personnel in Columbus, Ohio.

Since these reports were received, OSHA has monitored reports of accidents and injuries related to multi-piece rim wheels. In addition, petitions for the promulgation of a standard relating to the servicing of multi-piece rim wheels were submitted to OSHA in 1976 by the Rubber Manufacturers Association (RMA) and the Firestone Tire and Rubber Company. The National Highway Traffic Safety Administration (NHTSA), U.S. Department of Transportation (DOT), stated its support for the promulgation of such a standard and has, by written request, urged OSHA to regulate the servicing of multi-piece rim wheels in the workplace.

NHTSA is currently investigating the safety hazards associated with the use of multi-piece rims. It issued an advance notice of proposed rulemaking on March 5, 1979 (44 FR 12072) to determine whether to require certain performance levels for tire and rim component retention and whether to ban the production of multi-piece rims. NHTSA's actions are not directed at working conditions of employees and therefore are not an exercise of statutory authority by a federal agency under Section 4(b)(1) of the Occupational Safety and Health Act which would preempt action by OSHA.

NHTSA does not intend that its regulations displace OSHA's coverage of tire servicing personnel. NHTSA has articulated this intent by that agency's recognition that numerous accidents occur because of improper servicing, coupled with NHTSA's formal request that OSHA promulgate a standard for servicing of multi-piece rims in the workplace [Ex. 2: (30-17)].

On April 24, 1979, after a review of the available data, OSHA published a proposed permanent standard for the servicing of multi-piece rim wheels (44 FR 24252). The proposal contained requirements for training of all tire servicing employees, establishment of safe operating procedures for servicing multi-piece rim wheels, use of restraining devices and criteria for serviceability and interchangeability of rim components. A period for receipt of written comments on the proposed standard and issues raised therein was established, extending through July 6, 1979.

To assist participants in preparing their written comments and to give interested persons an opportunity to obtain clarification of the proposal, OSHA scheduled a public meeting for June 19, 1979, more than two weeks prior to the end of the comment period. During the meeting several participants submitted further comments on the proposed standard. A transcript of the meeting was prepared and is part of the record of this rulemaking.

Fifty-nine written comments were received by the end of the comment period. Most of the comments favored the adoption of the proposed standard in principle. A number of comments offered recommendations for minor modification of certain of the provisions of the proposal. There were no requests for a hearing under section 6(b)(3) of the OSHA Act.

A Regulatory Assessment was prepared in accordance with Executive Order 12044 (43 FR 12661, March 24, 1978), and was made available to the public, as noted in the preamble to the

proposed standard (44 FR 24246). (See Section D, Regulatory Assessment, below). Opportunity was given to interested persons to comment on the subject matter and contents of that report.

This final standard on servicing of multi-piece rim wheels is based on a full consideration of the entire record of the rulemaking proceeding including the materials relied on in the proposal, the transcript of the public meeting, and all written comments and exhibits received. All materials in the record are available for public review and copying at the OSHA Docket Office, Room S6212, U.S. Department of Labor, 3rd Street and Constitution Avenue, NW., Washington, D.C. 20210, telephone (202) 523–7894.

3. Hazards

Although accidents may occur at any time when handling multi-piece rims, the primary danger arises during the process of inflating the tire. An inflated tire is a high pressure vessel; for example, a popular size 10.00 x 20 tire when inflated at 105 pounds per square inch gauge (psig) (7.38 kg/cm²) creates a force in excess of 40,000 pounds (18,144 kg) against the rim flange. This force, according to test data provided by the Insurance Institute for Highway Safety (IIHS), accelerated a locking ring to 130 mph (209 km/hr) and raised a 215 pound (97.5 kg) anthropomorphic dummy 10 feet (3.05 m) upward from a wheel resting horizontally on the pavement.

The principal hazard in mounting, installing, storing, and handling multi-piece rim wheels arises when they are assembled together and the unit is inflated to its required pressure or beyond. If a component is not set or seated in its proper position in relation to the other components, the rings or the removable flanges may separate violently from the assembly. Such separation may cause lock rings, or other components to be hurled violently through the air, with the likelihood of striking a person and causing serious injury or death. Such accidents are most likely to occur while a tire that has just been mounted on a rim is being inflated or immediately after it has been inflated.

Accidents that have caused the greatest number of injuries appear to have been due to improper mounting, use of damaged parts, or mismatch of component parts. Accidents may also occur because of overinflating the tire or striking the lock rings or rims with a hammer. Many accidents appear to have resulted from a lack of knowledge on the part of the employee servicing the tire as to proper handling techniques and the dangers involved in servicing multi-piece rim wheels. In written comments, the State of North Carolina said, "A large portion of the accidents, injuries, and fatalities related to multi-piece rim wheels are traceable to untrained, inadequately trained, or improperly trained personnel." [Ex. 3: (21)]

4. Accident Data

Incidents which result in a serious or fatal injury to a mechanic engaged in servicing a multi-piece rim wheel often are only reported locally. Therefore, the data available is believed to be limited to only a portion of the total injuries and fatalities which occur. The May 1974 issue of "Learn and Live," a monthly publication of the Industrial Safety Division of the Florida Department of Commerce, reported that the fatality toll in Florida from servicing multi-piece rims had risen to eleven over a period of ten and one half years. By the end of 1978, the toll had risen to fifteen.

On September 28, 1973, NHTSA's Office of Defects Investigation issued a report on its investigation of multi-piece rim failures (ODI Case No. 215). This report covered 29 accidents due to improper assembly procedures that resulted in serious injury or a fatality, involving KB and KW type wheels. The report indicated that many of the shop personnel who worked with the multi-piece rims in question may not have been aware of all the safety precautions to be followed when mounting or demounting these wheels.

On December 21, 1973, NHTSA issued a report on its investigation of RH5° wheel failures (ODI Case No. 150) that included investigation of 81 incidents which resulted in serious injury or fatality to employees engaged in servicing these wheels. This report recommended several courses of action which included discontinuance of the manufacture of this type of wheel; development and distribution of a poster illustrating the safety precautions to be used during multi-piece rim wheel assembly; and development and distribution of a matching chart showing the compatibility of parts of multi-piece rim wheels produced by different manufacturers. (NHTSA developed a safety precautions chart, and multi-piece rim wheel matching chart, after their report was issued. The contents of these charts are utilized by OSHA in the training and servicing provisions of this final standard.)

In addition to the pre-1973 accident reports supplied in the NHTSA investigations OSHA's Office of Management Data Systems and Statistical Coordination received reports of 10 fatal accidents involving servicing of multi-piece rim wheels which occurred during 1976 and 1977. These data were compiled from workers' compensation reports from 10 states.

Data supplied by RMA which are listed in Table 3–2 of the Regulatory Assessment indicate that 13% of all multi-piece rim accidents result in fatalities, 63% result in injuries and property damage, and no-injury accidents constitute the remaining 24% of the 165 cases reported for the years 1972–1975. Similarly, IIHS data indicate that fatalities constitute 18%, injuries 67% and property damage and no-injury accidents represent the remaining 15% of the 241 cases reported for the years 1968–1977. Neither data base is considered totally representative of the nation because the actual number of split-rim accidents is not ascertainable, nor can the annual frequency of occurrence be predicted with a high degree of accuracy. Since the reported accidents do not represent a statistical sampling, but are only cases known to each organization, these numbers are considered to represent a lower limit of accident experience.

A review of accident descriptions provided by IIHS indicates that 53% of accidents under OSHA jurisdiction have occurred while the tire was being mounted/demounted, 31% while the wheel was being installed/removed and the remainder (16%) when the wheel was being handled or moved. Five of the 241 accidents that were evaluated, occurred while a safety cage or restraint was being used. A breakdown of the 16% category of accidents which occurred during handling indicates that numerous accidents occurred while moving an inflated tire in the service area, measuring tire pressure, removing the valve core or simply while an inflated tire was stored at rest. In some cases, multi-piece wheels being serviced exploded and either injured or killed experienced tire service personnel. However, it would appear that in many cases, these employees had never received any training, nor had they ever been informed of the inherent hazards and the safety practices to be followed.

Although the data presented may not be statistically representative of all multi-piece wheel accidents, they provide an insight into the relative frequency of fatalities and injuries. Injuries have not been classified into categories of severity, but an examination of IIHS accident reports suggests the existence of a very high proportion of fatalities and severe injuries, including many permanent disabilities.

Until now, there have been no specific OSHA general industry standards that apply to the handling and servicing of multi-piece rims. In the construction

safety and health standards, § 1926.600(a) requires that a tire rack, cage, or equivalent protection be provided and used when inflating tires on multi-piece rims. Section 1926.600 is not affected by the standard being published today.

B. Summary and Explanation of the Standard and Major Issues

The following section discusses the individual requirements of the multi-piece rim wheel standard, including analysis of the major issues raised during the proceeding, the record evidence and the policy considerations underlying the various provisions of the standard.

The final standard sets requirements for training of all tire servicing employees, safe practice and procedures and the use of restraining devices. These and other portions of the standard, including those on criteria for interchangeability of rim components have been revised and clarified from the proposal as described in detail below.

The language of the standard essentially follows that of the proposal except for revisions based on OSHA's review of the entire rulemaking record, including written comments and testimony submitted at the public meeting.

Virtually all persons who participated in the rulemaking by submitting comments and/or appearing at the public meeting agreed with OSHA's determination that the principal causes of accidents involving multi-piece rim wheel separations could be eliminated by proper training of employees, availability and utilization of restraining devices and necessary tools and equipment and adherence to recommended safe procedures.

(1) *Scope-paragraph (a).* This standard is intended primarily to provide protection to employees engaged in servicing of multi-piece rim wheels used on trucks, buses or other large vehicles. It applies also to the servicing and maintenance of all other multi-piece rim wheels, wherever they are used. Workplaces covered by the construction industry standards are subject to § 1926.600, and are not intended to be covered by the general industry standard published today.

The proposed standard would only have covered the servicing of rims 16 inches or greater in diameter. However, the rulemaking record clearly indicates that the danger of an unintended explosive separation of a multi-piece rim wheel exists for rims less than 16 inches as well.

The Michigan Department of Labor reported one fatality and three severe injuries which occurred when multi-piece rim wheels less than 16 inches (40.6 cm) in diameter were being serviced. [Ex. 3:(8)]. In addition, IIHS stated in its comments regarding the scope of the proposal that

> the concept that smaller multipiece [sic] rims are somewhat different, is generally not true. All multipiece [sic] rims depend upon the same balance of interlocking metal components. [Ex. 3: (23)]

Comments were submitted documenting the general use of smaller, multi-piece rim wheels on trailer which transport cars, livestock and furniture, as well as "bob-tailed" tractors used to haul mobile homes. [Ex. 3:(13)]. Several manufactures, including The National Wheel and Rim Association and Firestone Tire and Rubber Company, also recommended changes in the scope of the standard, based on the fact that 15 inch (38.1 cm) rims are used in significant numbers. [Ex. 3:(18); 3:(33); 3:(39)] Accordingly, the standard's scope has been modified to apply to the servicing of all multi-piece rim wheels without regard to their size, as along as they contain a lock ring or side ring. This provision reflects the determiniation that it is the assembly of multiple pieces and not the size of the wheel which is relevant to the explosion hazard.

Several comments recommended that the scope of the standard should include aircraft wheels, which consist of more than one piece. [Ex. 3:(5); 3:(6)] However, a review of manufactures' descriptive rim and wheel material and field visits to commerical and military airports have revealed that aircraft wheels are not similar to the "multi-piece rim wheels" covered by the standard. Aircraft wheels consist of a two-piece disk design with mounting bolts to hold the two halves together. [Ex. 3:(37)] They do not have locking rings, and do not use the air pressure of the tire to hold the rim components together. In addition, different tools and procedures are required for bolted wheels. Therefore, bolted wheels do not present the type or degree of explosion hazard addressed by this standard. [Ex. 3:(44)] This standard will only cover multi-piece rim wheels containing a lock ring, or side ring and base. In order to clarify the scope in this regard the proposed definition of multi-piece rim wheels is being revised in the final standard. (See discussion of paragraph (b) "Definitions", below).

(2) *Definitions-paragraph (b).* The definitions are stated as commonly used in the tire industry; however, some have been modified slightly to accommodate the regulatory nature of this standard.

Throughout the relevant literature, the term "mounting" has two different meanings. In one case, "mounting" a tire means assembling a tire with an appropriate rim and tube, while in the other case it means attaching a wheel to an axle. A review of nationwide accident reports indicates that the word "mounting" is used in both senses throughout the United States. For the purposes of this standard, OSHA uses the terms "mount and demount a tire" to mean the assembly and disassembly of a wheel and its components. "Install and remove a wheel" means to attach and remove an assembled wheel to/from a vehicle axle hub. This choice of definitions lessens the possibility of confusion associated with the "demounting a tire" vs. "dismounting a wheel" usage, while still conforming to NHSTA and tire manufacturer terminology. The term "dismounting" is not used in this standard, but is replaced with "removal."

In order to clarify the scope of the standard, as noted above, the proposed definition of a multi-piece rim wheel is being changed. As defined in the proposal, a multi-piece rim wheel is a vehicle wheel rim consisting of two or more parts, at least one of which is detachable, designed to hold the tire in place on the rim. To clarify that this standard does not cover the types of multi-piece rim wheels that are bolted together, [Ex. 3:(4); 3:(37)], this definition is revised in the final standard to read as follows:

> "Multi-piece rim" means a vehicle wheel rim consisting of two or more parts, one of which is a side or locking ring designed to hold the tire on the rim by interlocking components when the tube is inflated, regardless of the sizes of the component parts.

The proposed definition of a "rim manual" is being clarified and amended to provide that any manual which contains appropriate instructions and safety precautions from the manufacturer or other qualified organization is acceptable as a rim manual. OSHA agrees with the comments which stated that the definition in the proposal was too restrictive, since it might have been interpreted as being limited to a publication supplied directly by the manufacturer.

The term "charts" is used in the final standard instead of the term "wall charts" because of the many formats in which the necessary information may be found. In addition, because multi-piece rim wheels are serviced frequently at remote locations away from the employer's premises, the use of the term

"wall charts" might be confusing in instances where there are no "walls" in the service area.

The proposed definition of "wall charts" was limited to the DOT wall charts on matching rim components and on safety precautions, and other publications containing the same instructions as these two charts. The definition is revised in the final standard to clarify that the term includes all publications, whether or not published by DOT, which contain at a minimum the same instructions as the DOT publications, for the type of multi-piece rim wheel being serviced.

Several comments stated that the proposed definition of the "service area" was too narrow, in that it did not take into account the remote locations (away from the employer's premises) where multi-piece rim wheels are routinely and frequently serviced. OSHA recognizes that the servicing activity at these remote locations is at least as hazardous as at the employer's premises, that the operations conducted are essentially the same, and that the same training, tools and procedures are applicable. In view of the above, the final standard has been modified to define a service area as any location where a multi-piece rim wheel is serviced. OSHA recognizes that this change in the definition of service area may create a greater demand for portable restraining devices. However, such devices are readily available. [Ex. 2:(3); 3:(21); 3:(59)] (See Regulatory Assessment pp. 19–23.)

The term "trajectory path" has been redefined. A review of the accident reports has revealed that because of the nature of an explosive separation of a wheel, the direction of the separated rim components is not entirely predictable. Therefore, the proposed definition has been changed to indicate that the trajectory is the potential path a component may be expected to follow and that it may deviate from the perpendicular. Likewise Appendix A has been changed to reflect possible trajectories but in no way is meant to limit the trajectory to those illustrated.

The term "trajectory path" is being revised to "trajectory," since the added word "path" would be redundant.

(3) *Training—paragraph (c).* The standard requires every employee who services multi-piece rim wheels to be trained by the employer in proper techniques and practices applicable to the type of wheel being serviced. Training is required because many tire mechanics do not understand the potential danger involved in servicing multi-piece rim wheels, and because of the need to remind employees of the hazards and appropriate measures. The need for training is substantiated by a review of accident cases in which there appears to be a lack of knowledge of safe operating practices.

Firestone Tire and Rubber Company said, "In our view, training is the only method to ensure the safety of those who will be working with truck and bus tires and rims." [Ex. 3:(33)]

OSHA considers that training, in conjunction with the use of a restraining device and clip-on chuck, can contribute significantly to a reduction of accidents.

This standard does not specify the details of the training program, but simply requires the development and maintenance of employee proficiency in given elements of servicing. A mechanic's level of proficiency can be established by demonstration of his familiarity with and ability to use the information contained in the charts and in this standard.

The training provisions of the standard are stated in performance language, allowing the employer flexibility in complying with the requirement for training. This places the burden of providing adequate training and the responsibility for evaluating the employee's proficiency solely on the employer. Employees are adequately trained if they have thorough knowledge of and can apply the information contained in the charts and in this standard.

The proposal contained no explicit requirement that an employee who demonstrates his ability to service multi-piece rim wheels must maintain that ability. This omission is remedied in the final standard. It is clear that an employee must maintain his ability to service multi-piece rim wheels as long as he is involved in this work.

Virtually all of the comments concurred that proper training of employees is a necessary prerequisite to a safe operation. Goodyear Tire and Rubber Company stated that

> proper and thorough training is essential toward achieving a reduction in the rate of accidents. In view of the consequences of improper handling and technique, this training must be as specific as possible, and should be embodied within a well-defined procedure. [Ex. 3:(40)]

Others commented that specific training criteria be developed. Suggestions included on-the-job training; requiring a refresher course once a year; maintaining a record of training for each employee; and having employees sign a statement acknowledging receipt of this training. [Ex. 3:(18); 3:(21); 3:(25)]

OSHA has considered the fact that some employees may need relatively little training and practical experience to grasp the proper methods, techniques and practices and would need little or no periodic refresher training. Others may require additional initial training and periodic refresher training to retain their knowledge of safe methods and procedures.

In the final standard, the training requirement has been revised to assure that an employee receives sufficient training to enable him to safely perform the tasks which are involved in servicing multi-piece rim wheels. In addition to the initial training required in the proposal, the final standard places a continuing obligation on the employer to evaluate the capability of his employee and conduct additional training as necessary to assure that the employee maintains his competence at servicing multi-piece rim wheels. This not only insures that the initial training was effective, but also provides a means of determining the need for remedial or refresher training.

(4) *Tire servicing equipment—paragraph (d).* The unintended explosive separation of multi-piece rim wheel components is the primary cause of most occupational accidents associated with these wheels. A majority of the accidents under OSHA jurisdiction have occurred while the tire was being inflated following assembly. Accordingly, a significant reduction of injuries can be attained through use of a restraining device, such as a cage, specifically designed to protect employees from lethal airborne wheel components. An accepted practice for employee protection is to use a cage surrounding the wheel in such a manner as to prevent any wheel component from being hurled beyond the cage boundaries. The standard requires use of a restraining device while inflating a tire off the vehicle, except that a tire may be inflated to 3 psig (.21 kg/cm^2) without a restraining device for the sole purpose of seating the wheel components. (See discussion on safe operating procedures, paragraph (f)).

Due to the magnitude of forces associated with a wheel separation, strength requirements for restraining devices are necessary. Specifying these requirements necessitates knowing the amount of potential energy stored in the compressed air of a tire that will be transferred to the restraining device during a separation. For example, an analysis of high speed film in which the rim base gutter cone angle was machined to favor an explosive separation indicated that 8,200 ft.-lbs. (11,119 Joules) of energy was released when a 10.00 x 20 test tire was inflated to 105 psig (9.38 kg/cm^2). Calculations of the total pneumatic energy in the tire

indicated 75,000 ft.-lbs. (101,700 Joules) of available potential energy. After the energy transfer is determined for a particular size wheel, selection of an appropriate factor of safety will lead to a properly-designed restraining device for use with that wheel.

OSHA proposed that the generally accepted minimum factor of safety, 1.5 for machinery, be used for the largest wheel that a restraining device could hold. Several comments recommended that such design details for specific restraining devices be certified by professional engineers. [Ex. 3: (10); 3: (35); 3: (40);]. Another comment recommended that the design be specified only by the performance objective rather than by detailed design specifications which may become obsolete as technology changes. [Ex. 3: (47)]

The proposed requirement for restraining devices to be capable of withstanding a force of 150% of the maximum tire size that the device can hold is revised in the final standard. Although a safety factor is necessary, it does not appear practical to require an employer to have a cage designed to withstand an impact several orders of magnitude beyond that which would ever be encountered during its use. Some restraining devices are built so that their capacity (the size of tire capable of being held) is greater than the size tire actually used in the device. This is usually done to ease the job of manual tire handling by providing extra room within the device. If the device had to be strong enough to restrain the explosive force of any tire capable of being held in the device, as required in the proposal, the device might be unnecessarily heavy, thereby exposing the employee to other hazards during its manual handling. Since the device can be rated for a maximum size tire which provides a margin of safety, the final standard has been written to provide that the restraining device must be able to withstand at a minimum 150% of the force of an unexpected wheel separation for the tire being handled, whether or not that wheel is the maximum size the device can hold. This provides the same margin of safety (1.5) as proposed, but more accurately reflects the actual usage of the restraining device in applying that margin.

In its proposal OSHA proposed to permit use of machinery or equipment other than cages as restraining devices. At that time the agency solicited information as to the availability and effectiveness of such other types of restraining devices.

Several comments supported the effectiveness of the cage type restraining device, including the portable cages currently available, but stressed that the standard should not restrict technology in developing other methods of restraint. [Ex. 3: (18); 3: (33)]

Ten comments were received on the issue of whether hydraulic lift rails are adequate restraining devices. Four were totally opposed to the use of hoist rails, whereas the others stated that they could be used under certain limited circumstances.

Those that were opposed stated that the use of hoists for this purpose is not safe, and that a hoist rail would have to be extensively modified to be used effectively and would provide only limited opportunity for use. [Ex. 3: (21); 3: (25); 3: (47)] Those who said a hoist could be used under certain circumstances contended that a hoist rail is better than nothing, but emphasized that it should be used only if it meets acceptable standards, including adequate size, strength, location and positioning [Ex. 3: (40); 3: (47)].

OSHA recognizes that most hoist rails have not been designed or specifically modified for use as restraining devices and that they are therefore unacceptable for this purpose. However, the final standard is written as a performance standard so as not to restrict the use of any specific type of device, including hoist rails, provided that the device is specifically designed to restrain multi-piece rim wheels. Whichever device is used must be capable of restraining the components of a multi-piece rim wheel during explosive separation, and must meet the 150% margin of safety.

The standard prohibits the use of any restraining device with cracks in welds or components, or with bent or broken components, because such defects may cause equipment failure when subjected to dynamic loading. Stress concentrations around some cracks may cause the 1.5 factor of safety to be exceeded for the material; thus, if a cracked member of a restraining device is loaded to its original design value, the material at the apex of the crack may become overstressed, resulting in failure of the device.

The provisions for removal of damaged restraining devices from service are expanded in the final standard to establish additional, more specific criteria for such removal. OSHA has determined that there are defects other than cracks in welds or components which would also affect the ability of the restraining device to perform its intended function. Components which are broken or bent due to mishandling, abuse or a prior accident, or are excessively corroded (pitted) cannot be relied upon to perform their intended function. Therefore, the final standard requires that restraining devices with these defects be removed from service.

Many of the defects which would make the restraining device incapable of performing its function can be remedied or repaired once the device is out of service. However, it is essential that the repaired device be examined by a qualified person in order to assure that the device's restraining capability has not been impaired. To insure that the restraining device is capable of performing its intended function, the standard requires that, after repairs are made, the device must be checked and certified as meeting the strength requirements of paragraph (d)(1)(i) by the manufacturer or a registered professional engineer before being placed back into service. As the designer of such equipment, the manufacturer is capable of determining a satisfactory method of repair. In addition, since the laws of most jurisdictions regulating the registration of professional engineers set standards of conduct and require levels of competence, it has been determined that allowing certification either by a professional engineer or by the manufacturer will permit the use of repaired equipment while assuring that repairs do not compromise the strength of the restraining device.

As stated in the proposal, inflation of tires installed on vehicles presents another major safety hazard. Many of the comments expressed concern that a restraining device was needed which could be used both during inflation of tires installed on vehicles and during handling of a wheel after inflation, but before its installation on a vehicle.

Most of the comments which addressed this question indicated that there is no practical method to provide such protection on the vehicle. [Ex. 3: (18); 3: (25); 3: (33); 3: (35); 3: (40)]

Other comments also indicated that there is no satisfactory restraining device for use while transporting and storing tire and wheel assemblies. [Ex. 3: (21); 3: (35); 3: (39)].

As indicated by the North Carolina Department of Labor in their comments:

> Although separation may occur during handling and storage between the service and installation operations, it is rare, especially if proper servicing and inspection procedures are followed. [Ex. 3: (21)]

After careful consideration, OSHA has determined that there is no practical method available to restrain wheel components while tires installed on vehicles are inflated or between the

inflation of a demounted wheel and the time the wheel is installed on a vehicle. The most practical procedure to assure employee safety during that time period is simply for the employee to minimize his exposure to the trajectory. (As noted in paragraph (f), wheels that have been driven underinflated at 80% or less of their recommended pressure or which have obvious or suspected damage to the tire or wheel components must be deflated before removal.)

The final standard requires the use of a restraining device only during the inflation of an assembled wheel off the vehicle. In order to provide protection when multi-piece rim wheels are serviced on the vehicle, the standard also requires that the employer provide equipment such as the clip-on-chuck and sufficient length of hose which permits the employee to be clear of the possible trajectory of each wheel component during inflation. During inflation and all other operations involving multi-piece rim wheels, the standard also requires the employee to stay out of the trajectory, unless the employer can show that it is necessary for the employee to be in the trajectory to service the tire.

The requirement that charts and rim manuals be made available remains largely unchanged from the proposal. The availability of current charts and rim manuals will assure ready reference for tire mechanics encountering unusual situations or rim matching problems.

In the proposed standard OSHA raised the issue as to whether a warning label for multi-piece rims should be specified. Section 6(b)(7) of the Occupational Safety and Health Act addresses ". . . the use of labels, or other appropriate forms of warning . . ." associated with employee exposure to hazards. Lock rings, side rings and rim bases, because of their size and operational use, do not lend themselves to being labeled. The manufacturer's name, size, type of rim and manufacturing date are presently required to be on each multi-piece rim in accordance with Federal Motor Vehicle Safety Standard 120. This information is imprinted into the metal components; however, due to surface rust and mud, legibility is reduced commencing with the use of the wheel. In addition, rim manufacturers claim that stamping letters into rim components creates stress raisers, a hazard in itself, and recommend that the imprinting of rim components be minimized.

Warning labels are usually affixed to equipment presenting a particular hazard. However, in this case, the majority of comments felt that the use of a warning label or tag on wheel components is impractical and infeasible. [Ex. 3: (18); 3: (33); 3: (35); 3: (40); 3: (47)]. Other comments stated that warning labels would be unnecessary if the training of those servicing multi-piece rim wheels was adequate. [Ex. 3: (56)]

In light of the technical and practical problems as noted in the record, OSHA has concluded that warning labels should not be required in this standard. It is OSHA's feeling that the required training will identify the potential hazards and dangers of servicing multi-piece rim wheels and will reinforce the prescribed correct and safe procedures to be followed.

The proposed requirement which specified that proper tools be used for repair or servicing of wheels is being revised because of confusion as to what constitutes a proper tool. A review of several rim manuals has shown that each one contains lists or otherwise identifies the safest, most acceptable tools for use in servicing the particular multi-piece rim wheels covered in the manual. The final standard, therefore, requires that only tools listed or identified in the respective rim manuals be used for servicing.

(5) *Wheel component acceptability—paragraph (e).* The standard requires that wheel and rim components not be interchanged between different manufacturers' wheel models, except as provided on the charts.

The proposal would have required that side or lock rings that are bent out of shape, corroded or broken not be used and that they be removed from the service area, and that any rim component containing visible cracks be removed from use and discarded.

After review of the proposal, OSHA recognized that the criteria for rejection of wheel components due to "corrosion" were not clear. Many wheel components in use will exhibit some surface rust when exposed to the rigors of usage but there is little likelihood that this surface rust, when not on a mating surface of the rim, will adversely affect the performance of the wheel. However, if the parts become so rusted as to actually affect internal grains of the metal structure (pitting the metal surface), OSHA doubts the continued reliability of the component. Therefore, the final standard clarifies this point by prohibiting the use of components which are pitted by corrosion, bent out of shape, or broken.

Wheel components must be inspected prior to assembly. The final standard, as did the proposal, requires that the mating surfaces of the rim gutter, rings and tire must be free of any surface rust, scale or rubber build-up prior to assembly and inflation.

Although the proposal stated that damaged components, be removed from the service area, some comments did not feel that was sufficient [Ex. 3: (18); 3: (25); 3: (31); 3: (40)]. The final standard goes further and requires that once components are damaged so as to require their removal from service, they are to be rendered unusable and discarded. OSHA believes that this will eliminate the possibility of inadvertent substitution of one unserviceable part for another unserviceable part and that adherence to this procedure will significantly reduce the potential hazard in servicing multi-piece rims.

(6) *Safe operating procedures—paragraph (f).* The standard requires that every employer instruct all his employees engaged in servicing multi-piece rim wheels in the practices and procedures prescribed in these standards.

Paragraph (f)(2) of the proposal would have required all tires which were driven underinflated (presumably, even if only slightly underinflated) to be deflated to 10 psig (.70 kg/cm^2) or less before removal from the axle. However, an additional paragraph, (f)(10), stated that *all* tires must be deflated prior to removal from the vehicle axle. It is clear that these two conflicting paragraphs have caused confusion. [Ex. 3: (26); 3: (30); 3: (36); 3: (38); 3: (54)]. These provisions were drafted too broadly and did not properly reflect the agency's intent. The intent of the proposal was to provide that when tires are driven while underinflated to a point where damage to the tire and wheel may have occurred, or when such damage otherwise is known or suspected to exist, the tire must be completely deflated prior to removal of the wheel from the vehicle axle.

Several comments expressed concern that both wheels on a dual assembly would have to be deflated before removal, even if only one had been driven underinflated or exhibited damage, if the provisions of proposed paragraph (f)(10) were followed. [Ex. 3: (10); 3: (38); 3: (46)] OSHA agrees that this would be impractical and inefficient and in some cases would expose the employee to the unnecessary risk of deflating and inflating another multi-piece rim wheel.

The final standard has been clarified to state that total deflation and removal is required only for the servicing of installed multi-piece rim wheels which exhibit obvious or suspected damage to the tire or wheel components or which have been driven underinflated at 80% or less of their recommended pressure.

Accordingly, deflation of both tires on dual assemblies is required by the final standard only if both tires meet any of these conditions.

The proposed requirement for deflating a tire before demounting is amended in the final rule to specify that the deflation must be accomplished by removing the valve core. Removal of the valve core assures the complete deflation of the tire. If the valve is only pressed to release pressure, air may still remain in the tire. Removal of the valve core also allows the tube, in the event of localized deformation during the demounting process, to either exhaust or take in more air, thereby eliminating localized areas of pressure and stress on the multi-piece rim components. [Ex. 3: (20); 3: (39)]

The proposed requirement prohibiting employees from entering into the trajectory during deflation is changed in the final standard to provide that employees must remain out of the trajectory unless the employer can show that it is necessary for the employee to be in the trajectory to service the tire. It is recognized that removal of the valve core requires the employee to place his hand into the trajectory. Once the valve core is removed, the employee must stay completely out of the trajectory until the tire is completely deflated.

The proposed requirement that an employee not lean or rest his body or any equipment against the restraining device remains unchanged in the final. If a tire explodes within the restraining device, the suddenly-applied force exerted against the frame will immediately be transferred to the object or person resting against it. Except for the force absorbed by the containment of the exploding wheel components, the effects of the force upon the person or object leaning against the frame will be almost as severe as if the frame was not present. This process can be compared to a pool ball being hit by a fast moving cue ball. The energy of the rings is transferred to the tool, object or person leaning against the restraining device.

The proposed requirement that before assembly and inflation of the wheel and tire, a rubber lubricant shall be applied to the bead and rim mating surfaces to reduce sliding friction received little comment and remains unchanged in the final standard.

The proposed safe operating procedure to assure proper seating of the components has been carried forward in the final standard. After a tire has been inflated in a restraining device and while the tire is still so protected, the tire, rim and rings are to be inspected to make sure they are properly seated and locked. If further adjustment work on the rim or rings is necessary, the tire must be deflated before proceeding with any adjustment.

The proposed prohibition against hammering, striking or forcing wheel components while the tire is inflated was strongly supported by many participants in this rulemaking and therefore remains unchanged in the final standard. [Ex. 3: (23); 3: (25); 3: (31); 3: (50)]

The proposed requirement for the servicing of tires off the vehicle has been changed. The proposal would have allowed tires to be inflated to not more than 10 psig (.70 kg/cm ^{2}) outside the restraining device for the sole purpose of seating the tube, flap and tire, lock ring. This provision raised serious concern among public participants.

The Goodyear Tire and Rubber Company stated that 10 psig was more air than needed to perform the desired tasks and that a minimum amount of air (no more than 3 psig (.21 kg/cm^2)) should be put in a tire while the wheel is not in a safety cage. They stated that even 10 psig. (.70 kg/cm^2) is enough tire pressure to cause injury should a side ring not be properly seated. [Ex. 3: (40)]

The American Trucking Association also expressed concern that 10 psig (.70 kg/cm^2) was excessive and recommended a lower air pressure for seating. [Transcript (Tr): 13–14]

Based upon the concerns expressed in the comments, the allowable air pressure for seating the lock ring or rounding out the tube outside a restraining device has been reduced from the proposed 10 psig to 3 psig.

For pressures at or below 3 psig (.21 kg/cm^2), the danger of an explosive separation is minimal. The low risk of injury during the seating process must be compared to a higher risk of injury due to an explosive separation if the rings are not properly seated. When a tire is placed into a restraining device, the lock rings may slip out of the gutter, thus setting the stage for a subsequent explosive separation. Therefore, to assure proper seating of lock rings, the standard permits partial inflation outside of a restraining device, as noted earlier.

The proposal required that whenever any part of a rim base, rings or lugs is to be subjected to a high temperature heat source, such as from a welding or brazing torch, the tire must be completely deflated. This provision was intended to address those instances where heat is used to release components which are frozen due to age, rust, or defect. However, if this is done while the tire is inflated, an explosion may result because the heat increases the air pressure in the tire. [Ex. 3: (23); 3: (33)]

The practice of using heat on wheel components received serious criticism from RMA, Budd Company, and The National Wheel and Rim Association. These parties objected to any application of heat to a component, as it would have a detrimental effect on the strength, yield modulus and other characteristics of the metal. [Ex. 3: (18); 3: (25); 3: (31)]

OSHA recognizes that the application of heat may adversely affect the design and function of wheel components. There are alternative methods of releasing frozen lugs that are in general use in the industry such as penetrating oil or graphite solution. [Ex. 3: (23); 3: (25)] Therefore, the final standard has been revised to prohibit entirely the use of heat on wheel components.

The proposed requirement that mounted wheels with inflated tires be moved or stored so that the trajectory does not pass through a service area has been deleted from the final rule, based upon the comments which pointed out the infeasibility of complying with this requirement. [Ex. 3: (32); 3: (37); 3: (47)] Such a requirement would also necessitate that personnel be moved every time a tire is moved and is not feasible. It has therefore been deleted from the final standard.

C. Regulatory Assessment

In the preamble to the proposal, OSHA noted that a regulatory assessment, which was prepared for the agency by Centaur Management Consultants, Inc., was available for review and comment. This assessment, which was developed pursuant to Executive Order No. 12044 (43 FR 12661, March 24, 1978), and DOL implementing procedures (44 FR 5570, January 26, 1979), examined the effects of compliance with the proposed standard on cost, productivity, employment, critical materials, energy and market structure. The major findings of the "Economic Impact Statement/ Assessment for Multi-Piece Rim Assemblies" include the following:

—The population at risk is approximately 322,000 persons. These persons are employed in 102,500 workplaces in ten industry segments. These persons will benefit from a safer workplace as a result of the standard.

—From 1968 to 1975, the wheel and rim industry reported a total of 295 injuries resulting from multi-piece rim accidents. A minimum of 22 fatalities resulted from these accidents.

—Provisions of the standard resulting in economic impact include the use of

restraining devices, clip-on-chuck assemblies and training.

—Capital costs resulting from compliance with the standard total $8,342,000. Total annualized costs including capital and training costs are estimated to total $3,810,000.

—Cost, productivity, employment, critical materials, energy and market structure impacts were examined. No significant impacts on any of these areas were found to result from compliance with the standard.

Based on estimated sales of restraining devices over the past 10 years, 24% of stationary workstations presently use cages or racks and 72% of mobile workstations presently use portable safety racks. It is estimated that those establishments currently using restraining devices also use clip-on-chucks with in-line valves. Therefore, approximately 77,700 establishments will have to purchase at least a $134 cage and a $21 clip-on-chuck for compliance with this standard. Wall charts and rim manuals are free to tire assemblers; consequently, only administrative costs are involved in their acquisition. The cost of training employees should not exceed an hour of employee time plus corresponding instructor time.

The major benefit to be attained by complying with this regulation is a significant reduction in the number of fatalities and permanent injuries which occur while servicing multi-piece rim wheels each year.

The benefit derived from using the proposed training technique is that it is applicable to both experienced employees and new hires, and it is flexible because employers may choose to conduct group training classes rather than individual instruction. In general, increased productivity, reduced insurance premiums, reduced workers compensation payments and fewer product liability suits can be expected through compliance with this standard.

Opportunity was given to interested persons to comment on and testify concerning the contents of the report and related issues. Since OSHA received no comments regarding the regulatory assessment, the determination that the standard on multi-piece rim wheels is not a "major" action in terms of economic impact remains unchanged. Based on the record, OSHA also concludes that the standard is both economically and as technologically feasible.

D. Effective Date

Based on the information in the regulatory assessment, and in the absence of any contentions to the contrary, it is anticipated that employers will have little difficulty in obtaining restraining devices, clip-on-chuck assemblies or training materials. There should be no need for extended delay for employers to implement the provisions of the standard. Therefore, the effective date of this standard is April 28, 1980.

E. Appendices

Two appendices have been included in this permanent standard for information purposes. Nothing contained in the appendices should be construed as establishing a mandatory requirement not otherwise imposed by the standard, or as detracting from an obligation which the standard does impose.

The information contained in Appendix A illustrates possible trajectories of wheel components during an explosive separation. Appendix B contains information concerning ordering of wall charts.

The contents of the proposed appendices have been clarified where necessary.

F. Authority

This document was prepared under the direction of Eula Bingham, Assistant Secretary of Labor for Occupational Safety and Health, U.S. Department of Labor, Third Street and Constitution Avenue, NW., Washington, D.C. 20210.

Accordingly, pursuant to section 6(b) of the Occupational Safety and Health Act of 1970 (84 Stat. 1593; 29 U.S.C. 655) Secretary of Labor's Order No. 8–76 (41 CFR 25059), and 29 CFR part 1911, Part 1910 of Title 29, Code of Federal Regulations is amended by adding a new § 1910.177 as set forth below.

Signed at Washington, D.C., this 18th day of January 1980.

Eula Bingham,

Assistant Secretary of Labor.

PART 1910—OCCUPATIONAL SAFETY AND HEALTH STANDARDS

A new § 1910.177 is added to 29 CFR Part 1910, to read as follows:

§ 1910.177 Servicing multi-piece rim wheels.

(a) *Scope.* This section applies to the servicing of vehicle wheels which have tube-type tires mounted on multi-piece rims as defined below in paragraph (b) of this section.

(b) *Defintions.* "Charts" means the United States Department of Transportation, National Highway Traffic Safety Administration (NHTSA) publications entitled "Safety Precautions for Mounting and Demounting Tube-Type Truck/Bus Tires" and "Multi-Piece Rim/Wheel Matching Chart," or any other publications containing, at a minimum, the same instructions, safety precautions and other information contained on those charts that are applicable to the types of multi-piece rim wheels being serviced.

"Installing a Wheel" means the transfer and attachment of an assembled wheel onto a vehicle axle hub. "Removing" means the opposite of installing.

"Mounting a Tire" means the assembly or putting together of rim components, tube, liner (flap) and tire to form a wheel, including inflation. "Demounting" means the opposite of mounting.

"Multi-piece rim" means a vehicle wheel rim consisting of two or more parts, one of which is a side or locking ring designed to hold the tire on the rim by interlocking components when the tube is inflated, regardless of the sizes of the component parts.

"Restraining device" means a mechanical apparatus such as a safety cage, rack, or safety bar arrangement or other machinery or equipment specifically designed for this purpose, that will constrain all multi-piece rim wheel components following their release during an explosive separation of the wheel components.

"Rim manual" means a publication containing instructions from the manufacturer or other qualified organization for correct mounting, demounting, maintenance and safety precautions peculiar to the multi-piece rim being serviced.

"Service" or "servicing" means the mounting and demounting of multi-piece rim wheels, and related activity such as inflating, deflating, installing, removing, maintaining, handling or storing of multi-piece rim wheels, including inflating and deflating of wheels installed on vehicles.

"Service area" means that part of an employer's premises used for the servicing of multi-piece rim wheels, or any other place where an employee services multi-piece rim wheels.

"Trajectory" means any potential path or route that a lock ring, side ring, rim base and/or tire may travel during an explosive rim separation, and includes paths which may deviate from that perpendicular to the assembled position of the components on the rim base at the time of separation. (See Appendix A for examples of expected trajectories).

"Wheel" means an assemblage of tire, tube, and multi-piece rim components.

(c) *Employee training.* (1) The employer shall provide a training

program to train and instruct all employees who service multi-piece rim wheels in the hazards involved in servicing multi-piece rim wheels and the safety procedures to be followed.

(i) The employer shall assure that no employee services any multi-piece rim wheel unless the employee has been trained and instructed in correct procedures of mounting, demounting, and all related services, activities, and correct safety precautions for the rim type being serviced, and the safe operating procedures described in paragraph (f) of this section.

(ii) Information to be used in the training program shall include, at a minimum, the data contained on the charts and the contents of this standard.

(iii) Where an employer knows or has reason to believe that any of his employees is unable to read and understand the charts or rim manual, the employer shall assure that the employee is instructed concerning the contents of the charts and rim manual in a manner which the employee is able to understand.

(2) The employer shall assure that each employee demonstrates and maintains his ability to service multi-piece rim wheels safely, including performance of the following tasks:

(i) Demounting of tires (including deflation);

(ii) Inspection of wheel components;

(iii) Mounting of tires (including inflation within a restraining device);

(iv) Use of the restraining device;

(v) Handling of wheels;

(vi) Inflation of tires when a wheel is mounted on the vehicle; and

(vii) Installation and removal of wheels.

(3) The employer shall evaluate each employee's ability to perform these tasks and to service multi-piece rim wheels safely and shall provide additional training as necessary to assure that each employee maintains his proficiency.

(d) *Tire servicing equipment.* (1) The employer shall furnish and shall assure that employees use a restraining device in servicing multi-piece rim wheels.

(i) Each restraining device shall have the capacity to withstand the maximum force that would be transferred to it during an explosive wheel separation occurring at 150 percent of maximum tire specification pressure for the wheels being serviced.

(ii) Restraining devices shall be capable of preventing rim components from being thrown outside or beyond the frame of the device for any wheel position within the device.

(iii) Restraining devices shall be inspected prior to each day's use and after any explosive separation of wheel components and any restraining devices exhibiting any of the following defects shall be immediately removed from service:

(A) cracks at welds;

(B) cracked or broken components;

(C) bent or sprung components caused by mishandling, abuse or wheel separation; or

(D) pitting of components due to excessive corrosion.

(iv) Restraining devices removed from service in accordance with paragraph (d)(1)(iii) of this section, shall not be returned to service until they are inspected, repaired, if necessary, and are certified either by the manufacturer or by a Registered Professional Engineer as meeting the strength requirements of paragraphs (d)(1) (i) and (ii) of this section.

(2) A clip-on-chuck with a sufficient length of hose to permit the employee to stand clear of the potential trajectory of the wheel components, and an in-line valve with gauge or a pressure regulator preset to a desired value shall be furnished by the employer and used to inflate tires.

(3) Current charts shall be available in the service area.

(4) A current rim manual containing instructions for the type of rims being serviced shall be available in the service area.

(5) The employer shall assure that only tools recommended in the rim manual for the type of wheel being serviced are used to service multi-piece rim wheels.

(e) *Wheel component acceptability.* (1) Wheel components shall not be interchanged except as provided in the charts, or in the applicable rim manual.

(2) Wheel components shall be inspected prior to assembly. Rim bases, side rings or lock rings which are bent out of shape, pitted from corrosion, broken or cracked shall not be used and shall be rendered unusable and discarded.

(3) Mating surfaces of the rim gutter, rings and tire shall be free of any dirt, surface rust, scale or rubber buildup prior to mounting and inflation.

(f) *Safe operating procedure.* The employer shall establish a safe operating procedure for servicing multi-piece rim wheels and shall assure that employees are instructed in and follow that procedure. The procedure shall include at least the following elements:

(1) Tires shall be completely deflated before demounting by removal of the valve core.

(2) Tires shall be completely deflated by removing the valve core, before a wheel is removed from the axle in either of the following situations:

(i) When the tire has been driven underinflated at 80% or less of its recommended pressure, or

(ii) When there is obvious or suspected damage to the tire or wheel components.

(3) Rubber lubricant shall be applied to bead and rim mating surfaces during assembly of the wheel and inflation of the tire.

(4) Tires shall be inflated only when contained by a restraining device, except that when the wheel assembly is on a vehicle, tires that are underinflated but have more than 80% of the recommended pressure, may be inflated while the wheel is on the vehicle if remote control inflation equipment is used and no employees are in the trajectory, and except as provided in paragraph (f)(5) of this section.

(5) When a tire is being partially inflated without a restraining device for the purpose of seating the lock ring or to round out the tube, such inflation shall not exceed 3 psig (0.21 kg/cm^2).

(6) Whenever a tire is in a restraining device the employee shall not rest or lean any part of his body or equipment on or against the restraining device.

(7) After tire inflation, the tire, rim and rings shall be inspected while still within the restraining device to make sure that they are properly seated and locked. If further adjustment to the tire, rim or rings is necessary, the tire shall be deflated by removal of the valve core before the adjustment is made.

(8) No attempt shall be made to correct the seating of side and lock rings by hammering, striking or forcing the components while the tire is pressurized.

(9) Cracked, broken, bent or otherwise damaged rim components shall not be reworked, welded, brazed, or otherwise heated.

(10) Whenever multi-piece rim wheels are being handled, employees shall stay out of the trajectory unless the employer can demonstrate that performance of the servicing makes the employee's presence in the trajectory necessary.

BILLING CODE 4510-26-M

APPENDIX A
TRAJECTORY

WARNING

STAY OUT OF THE TRAJECTORY AS INDICATED BY SHADED AREA

Note: Under some circumstances, the trajectory may deviate from its expected path

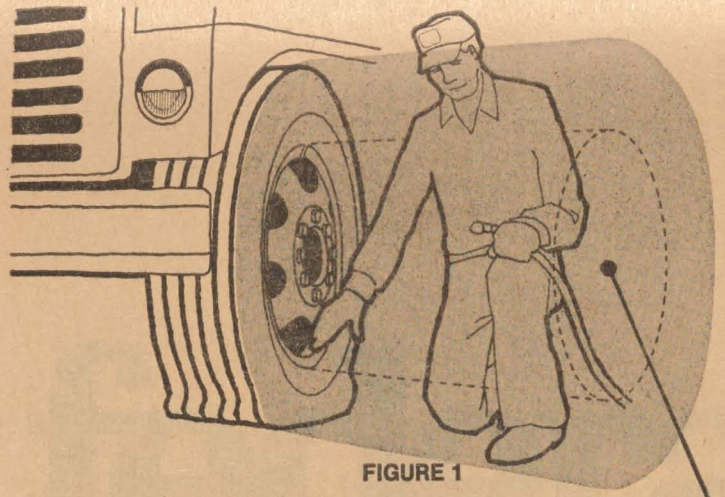
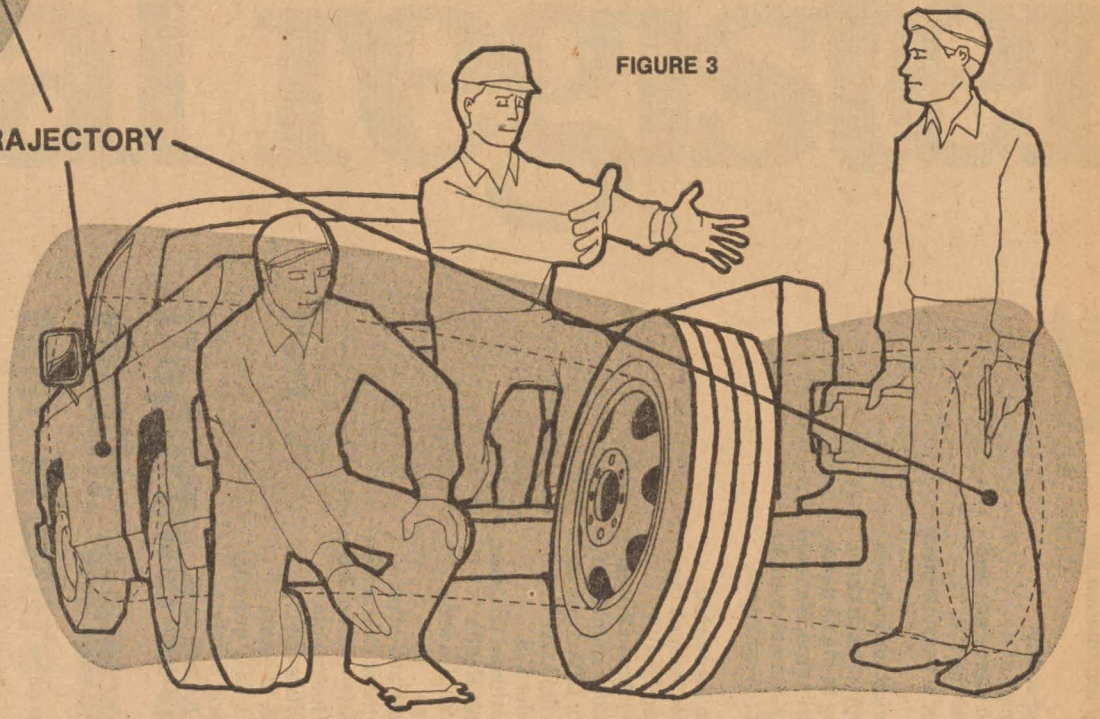


FIGURE 1

FIGURE 2

TRAJECTORY

FIGURE 3



BILLING CODE 4510-26-C

Appendix B—Ordering Information for NHTSA Charts

NHTSA has prepared safety information charts as part of a continuing campaign to alert truck and bus service personnel to the risk involved when working with multi-piece truck and bus wheels.

Individuals who service such wheels may obtain a single copy of each chart, without cost, by writing to the General Services Division/Distribution, National Highway Traffic Safety Administration, 400 Seventh Street SW., Washington, D.C. 20590.

Reprints of the above mentioned charts are also available through the Occupational Safety and Health Administration (OSHA) Area Offices. The address and telephone number of the nearest OSHA Area Office can be obtained by looking in the local telephone directory under U.S. Government, U.S. Department of Labor, Occupational Safety and Health Administration. Single copies are available without charge.

Service establishments and other organizations desiring these charts may order them in any quantity desired from the Superintendent of Documents, Government Printing Office (GPO), Washington, D.C. 20402, at a cost established by the GPO. GPO ordering number for the charts are: Safety Chart—050–003–00315–8, Cost: $2.25, Matching Chart—050–003–00316–6, Cost: $2.00.

(Sec. 6, 84 Stat. 1593 (28 U.S.C. 655); Secretary of Labor's Order 8–76 (41 FR 25059), 29 CFR Part 1911.)

[FR Doc. 80–2768 Filed 1–28–80; 8:45 am]

BILLING CODE 4510–26–M

federal register

Tuesday
January 29, 1980

Part V

Department of Health, Education, and Welfare

National Institutes of Health

Recombinant DNA Research; Actions Under Guidelines

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

National Institutes of Health

Recombinant DNA Research; Actions Under Guidelines

AGENCY: National Institutes of Health, PHS, HEW.

ACTION: Notice of actions under NIH Guidelines for Research Involving Recombinant DNA Molecules.

SUMMARY: This notice sets forth actions taken by the Director, NIH, under the 1978 NIH Guidelines for Research Involving Recombinant DNA Molecules (43 FR 60108). Revised NIH Guidelines are printed following this notice.

EFFECTIVE DATE: January 29, 1980.

FOR FURTHER INFORMATION CONTACT: Additional information can be obtained from Dr. William J. Gartland, Office of Recombinant DNA Activities (ORDA), National Institutes of Health, Bethesda, Maryland 20205. (301) 496–6051.

SUPPLEMENTARY INFORMATION: I am promulgating today revised NIH Guidelines for Research Involving Recombinant DNA Molecules. This announcement is a "Decision Document" explaining the background and reasons for my decision. Immediately following this announcement there appears a copy of the revised NIH Guidelines.

The structure of this Decision Document is as follows:

I. *History of the NIH Guidelines Through 1978.*

II. *Revision of the December 1978 Guidelines.*

III. *Analysis of Comments on Decision Document/Environmental Impact Assessment/Proposed Revised Guidelines as Published For Comment in the Federal Register on November 30, 1979 (44 FR 69210).*

I. History of the NIH Guidelines Through 1978

The history leading to the issuance of the original 1976 NIH Guidelines for Recombinant DNA Research is described in detail in the Environmental Impact Statement on the 1976 Guidelines, and in the "Decision Document" accompanying the Guidelines in the **Federal Register** of July 7, 1976. Key points in the history included:

- The Maxine Singer-Dieter Soll letter (*Science 181*, 1114, 1973) arising from the Gordon Research Conference on Nucleic Acids of July 1973.
- The Paul Berg et al. letter to *Science (185*, 303, 1974) calling for the NIH to establish an advisory committee to write guidelines.
- The Asilomar conference of February 1975.
- The work of the NIH Recombinant DNA Advisory Committee (RAC) through 1975, resulting in the proposed guidelines of December 1975.
- The special meeting of the Advisory Committee to the Director, NIH, on February 9–10, 1976, to review the proposed guidelines.
- Final issuance of the NIH Guidelines on June 23, 1976 (published in the **Federal Register** on July 7, 1976).

The history from the period July 1976 to December 1978 included the following key points:

- Deliberations on revisions by the RAC during 1977, resulting in proposed revisions published for comment in the **Federal Register** on September 27, 1977 (42 FR 49596).
- A public hearing on the revisions, at the meeting of the Advisory Committee to the Director, NIH, December 15–16, 1977.
- Publication for public comment in the **Federal Register** on July 28, 1978 (43 FR 33042), of new proposed revised guidelines accompanied by a detailed Decision Document and a detailed Environmental Impact Assessment.
- A public hearing on the proposed revisions, chaired by the General Counsel of HEW, on September 15, 1978.
- Publication of revised guidelines on December 22, 1978 (43 FR 60080), accompanied by a detailed Decision Document and Environmental Impact Assessment.

The entire history is extensively documented in Volumes 1–4 of "Recombinant DNA Research"—a series constituting a readily available public record of activities in regard to the NIH Guidelines.

II. Revision of the December 1978 Guidelines

The December 1978 NIH Guidelines for Research Involving Recombinant DNA Molecules (43 FR 60108) include procedures for changing the Guidelines. As detailed in Section IV–E–1–b–(1) of the Guidelines, this involves: (1) publication in the **Federal Register** for public comment, at least 30 days prior to a RAC meeting, of the proposed changes; (2) consideration of the proposed changes by the RAC; and (3) publication in the **Federal Register** of the final decision by the Director, NIH.

In accordance with these procedures, proposed changes in the Guidelines appeared in the **Federal Register** on January 15, 1979 (44 FR 3226), were considered by the RAC at its February 16–17, 1979, meeting, and were promulgated by the NIH Director in the **Federal Register** on April 11, 1979 (44 FR 21730).

Proposed changes in the Guidelines appeared in the **Federal Register** on April 13, 1979 (44 FR 22314), were considered by the RAC at its May 21–23, 1979, meeting, and were promulgated by the NIH Director in the **Federal Register** on July 20, 1979 (44 FR 42914).

Proposed changes in the Guidelines appeared in the **Federal Register** on July 31, 1979 (44 FR 45088) and were considered by the RAC at its September 6–7, 1979, meeting. Rather than promulgating the recommended changes, the Director, NIH, instead issued them for 30 days of additional public comment in the **Federal Register** on November 30, 1979 (44 FR 69210).

This was in accordance with Section IV–E–1–b–(1) of the NIH Guidelines (43 FR 60126) which says, "The Director's proposed decision, at his discretion, may be published in the **Federal Register** for 30 days of comment before final action is taken." What appeared in the **Federal Register** on November 30 was a detailed "Decision Document" explaining the background and reasons for the proposed decision, an Environmental Impact Assessment, and proposed revised NIH Guidelines. Included was an analysis of many letters received prior to November 30. Part III of the present document contains an analysis by the Director, NIH, of all comments received during the period November 30, 1979 to January 18, 1980 on the Decision Document/Environmental Impact Assessment/Proposed Revised Guidelines as published in the **Federal Register** on November 30, 1979. All of the changes in the Guidelines accepted by the Director, NIH, and promulgated today have been found by the Director, NIH, in accordance with Section IV–E–1–b of the NIH Guidelines, to comply with the Guidelines and to present no significant risk to health or the environment.

Proposed changes in the Guidelines appeared in the **Federal Register** on November 1, 1979 (44 FR 63074), were considered by the RAC at its December 6–7, 1979, meeting, and were promulgated by the NIH Director in the **Federal Register** on January 17, 1980 (45 FR 3552).

Immediately following this "Decision Document," there appears a copy of the revised NIH Guidelines which are effective today. These were obtained by incorporating into the December 1978 Guidelines all the changes made following the February 16–17, 1979, May 21–23, 1979, September 6–7, 1979, and December 6–7, 1979, RAC meetings.

III. Analysis of Comments on Decision Document/Environmental Impact Assessment/Proposed Revised Guidelines as Published for Comment in the Federal Register on November 30, 1979 (44 FR 69210)

III–A. Discussion at RAC Meeting on December 6, 1979

The Decision Document/ Environmental Impact Assessment/ Proposed Revised Guidelines as sent to the **Federal Register** to appear on November 30, 1979, were simultaneously sent to RAC members who received the documents on November 29. The material was discussed by the RAC at its December 6–7, 1979, meeting. At this meeting, the RAC Chairman presented the document pointing out the changes between the "*E. coli* K–12/P1 Recommendation" as adopted by the RAC on September 6, 1979, and the somewhat revised version of this recommendation (Section III–O of the proposed revised Guidelines) as issued by the NIH Director for public comment in the **Federal Register** on November 30, 1979. She noted that the NIH Director had eliminated the reference to these experiments as "exempt from the Guidelines" and had added a requirement for prior review and approval by the IBC for experiments in which there is a deliberate attempt to have the *E. coli* K–12 efficiently express a gene coding for a eukaryotic protein. The Chairman asked for comments from the RAC. Except for questions of clarification from RAC members, which were answered by NIH staff, there were no comments either on these particular items or on the recommendations generally. NIH staff urged RAC members to write individually to the NIH Director during the comment period giving their views. (Six RAC members did write. Four endorsed Section III–O of the Guidelines. Two, who had voted against the "*E. coli* K–12/P1 Recommendation" at the September 6–7, 1979, meeting, wrote. One urged the "exemption" not be approved. The other urged that the final decision not be delayed.)

III–B. Public Comments

The Decision Document/ Environmental Impact Assessment/ Proposed Revised Guidelines as they appeared in the **Federal Register** on November 30, 1979, were sent out to over 2000 people for comment—this included the chairmen of all Institutional Biosafety Committees registered with NIH, all principal investigators doing recombinant DNA experiments supported by NIH, and all persons who had previously requested their inclusion on a mailing list to receive information concerning the NIH Guidelines. During the period up to January 18, 1980, 185 letters signed by a total of 205 individuals were received. All of these letters: (i) are now available for public inspection at ORDA; (ii) can be made available (in whole or in part) to any requester upon payment of reproduction costs; and (iii) will be published (and subsequently may be purchased through the U.S. Government Printing Office) as part of Volume 5 of "Recombinant DNA Research," a series constituting a public record of activities in regard to the NIH Guidelines.

The Decision Document/ Environmental Impact Assessment consisted of an analysis of the six "major actions" which were recommended favorably at the September 6–7, 1979, RAC meeting. These six "major actions" were: "The *E. coli* K–12/P1 Recommendation"; "Proposed Amendment of Sections II–D–1–a–(1) and III–A–1–b–(1) of the Guidelines"; "Proposed Exemption for *Pseudomonas Putida* and *Pseudomonas Florescens*"; "Cloning in *Bacillus Subtilis* and *Streptomyces Coelicolor*"; "Use of *Agrobacterium Tumefaciens* as a Host-Vector System"; and "Proposed Supplement (Part VI) to the Guidelines."

The bulk of the November 30 Decision Document/Environmental Impact Assessment consisted of an analysis of the "*E. coli* K–12/P1 Recommendation"; it was ponted out that "of all the recommendations arising from the last three meetings of the RAC [this recommendation was] the one that has generated the greatest number of letters and the most discussion at the RAC meetings." The analysis included the NIH Director's proposed acceptance of a modified version of this recommendation to become Section III–O of the proposed revised Guidelines.

In the comment period only three letters were received that included comments dealing specifically with any of the other five "major actions" i.e., all other letters made reference to the entire proposed revised Guidelines or commented specifically upon the "*E. coli* K–12/P1 Recommendation." The remainder of this document is organized as follows: III–B–1. Comments on The Entire Proposed Revised Guidelines; III–B–2. Comments on the "*E. coli* K–12/P1 Recommendation" or Section III–O of the Proposed Revised Guidelines; III–B–3. Comments on the Proposed Revised Guidelines Other Than Section III–O; III–B–4. Comments on the Guidelines Other Than Changes Recommended by the RAC; III–C. Decision of the NIH Director on Promulgation of Revised Guidelines.

III–B–1. Comments on the Entire Proposed Revised Guidelines

Eighty-three letters signed by a total of 100 individuals were received in support of the proposed revised Guidelines. (Many of these commentators also specifically endorsed Section III–O of the proposed Guidelines.) Comments included the following—"I heartily support the changes that you propose for the NIH Guidelines for Research Involving Recombinant DNA Molecules. I am especially impressed by the detailed and reasoned consideration that the Advisory Committee (RAC) and you have used to reach these very enlightened decisions."—"This letter is to indicate my wholehearted support of the revisions."—"Although I am highly concerned with laboratory safety, I believe the revised guidelines are certainly reasonable."—"They are reasonable and sensible Guidelines which take into account the body of new information and research experience which has become available since the formulation and enactment of the original guidelines."—"The proposed new Guidelines are a very sensible step forward. By freeing scientists from unnecessary red tape, and administrative delays in doing experiments, they will appreciably accelerate the progress of research and the realization of its benefits."

III–B–2. Comments on the "E. coli K–12/P1 Recommendation" or Section III–O of the Proposed Revised Guidelines

Comments received on the RAC's "*E. coli* K–12/P1 Recommendation" or the NIH Director's proposed incorporation of a modified version of this recommendation to become Section III–O of the proposed revised Guidelines are discussed below.

III–B–2–a. Endorsement of the "E. coli K–12/P1 Recommendation" or Section III–O of the Proposed Revised Guidelines

In addition to the 83 letters mentioned above which endorsed the entire proposed revised Guidelines, another 86 letters signed by a total of 89 individuals were received endorsing what was referred to as either the proposed new "Section III–O of the Guidelines," the "*E. coli* K–12/P1 Recommendation," or the "decision to reclassify recombinant DNA experiments performed in *E. coli* K–12 as P1." Thus, of the 185 letters received, 169 supported the proposed new Section III–O.

These commentators included four RAC members, and six former RAC members. Comments included the following—"Section III–O describing experiments with *E. coli* K–12 host-vector systems represents a realistic and safe modification of some of the previous regulations. . . . We wish to express our confidence in the good judgment and scientific qualifications of the committee that has made these decisions. The enormous effort in preparing these guidelines in the interest of all of us should earn high praise."—"In Section III–O there is a classificatory downgrading of a large group of experiments in *E. coli* K–12. I applaud that change. It appears to me to be soundly based on the accumulating experience and evaluation of real hazards of such experiments."—"For this reason, I strongly endorse the decentralization of control over experiments using the *E. coli* K–12 Host-Vector systems as outlined in section III–O."—"I, therefore, urge adoption of Section III–O, as a way of eliminating a costly and time-consuming unnecessary obstacle to research of great practical importance as well as scientific interest."—"In particular, I specifically endorse the revision of the guidelines concerning the K–12 containment (section III–O). The proposals are a reasonable way of matching the realistic risks with the clear benefit of removing unnecessary administrative work."—"I believe that the category change is fully consistent with public safety, and is essential to permit legitimate health related research dependent upon cloning techniques to proceed."—"I consider the evidence overwhelming that these experiments pose no significant hazard."

III–B–2–b. Request that Experiments Involving E. coli K–12 Be Exempted from the Guidelines

Nine commentators, while indicating their endorsement of Section III–O, also indicated that they favored a somewhat greater relaxation of the Guidelines. Comments included the following—"My personal opinion is that the data does not even warrant registration of these experiments."—"My current view is that even P1 containment is probably unnecessary."

Nineteen commentators wrote requesting that all or most experiments with *E. coli* K–12 be completely exempted from the Guidelines; this would relax the conditions for doing these experiments much further than I had proposed in Section III–O of the proposed revised Guidelines. (Some of these commentators endorsed Section III–O as a "step in the right direction.") Comments included the following—"To continue Federal regulation after evidence has been obtained that there is no clear threat to the public health is a waste of already dwindling Federal scientific resources and in addition, sets an ominous precedent for future Federal regulatory adventures. In addition, at the level of the working scientist or student, the perpetration of needless regulations, directed at imagined hazards, undercuts our continuing efforts to institute and make effective safety practices governing the handling of real pathogens and toxic agents."

On the other hand, four commentators specifically endorsed the decision that experiments with *E. coli* K–12 not be exempted from the Guidelines, and four commentators specifically endorsed IBC registration of these experiments.

In my Decision Document/Environmental Impact Assessment of November 30, 1979, I discussed why I was not proposing to exempt from the Guidelines experiments under the "*E. coli* K–12/P1 Recommendation." As I wrote then, and still believe is prudent policy: "Three important safety features for these experiments that will not be exempt, but will according to the proposed decision form a special class under the Guidelines, are:

"1. P1. Containment—Including the ban on mouth pipetting and the requirement that all biological wastes shall be decontaminated. Proper employment of P1 conditions eliminates the primary means of *E. coli* escape from the laboratory.

"2. EK1—Allowing only *E. coli* K–12 strains and not allowing the use of conjugation proficient plasmids or generalize transducing phages. This greatly reduces the probability that any escaping *E. coli* K–12 would survive and transfer their recombinant DNA to other organisms.

"3. IBC Oversight—Continuing local surveillance and registration of these experiments.

"In addition, keeping these experiments under the Guidelines rather than exempting them means that any scale-up of the experiments beyond 10 liters will require prior NIH approval."

These important safety features apply to the experiments described in Section III–O of the proposed revised Guidelines; they would not apply if these experiments were exempted from the Guidelines.

III–B–2–c. Request That Section III–O of the Guidelines Not Be Promulgated

Of the 185 letters received by January 18, 1980, five said that Section III–O and/or the proposed revised Guidelines should not be promulgated. These five commentators included one current and one former RAC member. Among the comments they made were:

1. "I urge you to extend the comment period."

2. The NIH Director should reconsider "the *E. coli* exemption as voted for by the RAC at its September 1979 meeting."

3. "It needs emphasis that there is currently no *requirement* (only a recommendation) that institutions require workers in this field to be trained in good laboratory practice."

4. Many of the arguments used to justify this revision of the Guidelines were used to justify a previous revision.

5. The discussion in the November 30 Decision Document "implies that microorganisms do not 'escape' from laboratories in which containment is supposed to be practiced."

6. "I continue to be disturbed that such far-reaching policy changes are being considered *in the absence of data from a risk-assessment program.*"

7. "I'm less than totally convinced by the information in the November 30, 1979 **Federal Register** that it is prudent to allow cloning of all DNA at the P1 + EK1 level except where prohibited."

None of these commentators provided any new scientific data.

In reply to the first comment given above, I note that although the comment period formally ended December 30, 1979, I considered all letters received until January 18, 1980.

In response to the second comment given above, I note that the November 30 Decision Document/Environmental Impact Assessment discussed in detail why I am not in fact exempting these experiments from the Guidelines.

In response to the third comment given above, the NIH Guidelines do in fact require training of workers. Section IV–D–1–g of the Guidelines discussing responsibilities of the Institution says the Institution shall "Ensure appropriate training for the IBC chairperson and members, the BSO, Principal Investigators (PIs), and laboratory staff regarding the Guidelines, their implementation, and laboratory safety. Responsibility for training IBC members may be carried out through the IBC chairperson. Responsibility for training laboratory staff may be carried out through the PI. The Institution is responsible for seeing that the PI has sufficient training, but may delegate this responsibility to the IBC." Section IV–D–3–a–2 says the Institutional Biosafety Committee is responsible for "An assessment of the facilities, procedures, and practices, and of the training and expertise of recombinant DNA personnel." Section IV–D–5–d–2 of the Guidelines says the Principal Investigator is responsible for

"Instructing and training staff in the practices and techniques required to ensure safety and in the procedures for dealing with accidents."

In response to the fourth comment given above, I note that this was discussed in the November 30 Decision Document/Environmental Impact Assessment under the consideration of the comments "Data Are Not Sufficient To Justify Exemption."

In response to the fifth comment given above, we did not mean to imply that microorganisms do not escape from laboratories in which containment is practiced. Data on laboratory-acquired infection rates at different physical containment levels were given in the NIH Environmental Impact Statement on the 1976 Guidelines where it was pointed out that "when known hazardous agents are handled, the risk of a laboratory-acquired infection cannot be totally eliminated." What is discussed in the November 30, 1979, Decision Document/Environmental Impact Assessment is the low probability of *E. coli* K–12 escaping in significant numbers from a P1 laboratory. This, combined with the low probabilities of a series of other steps discussed in that document, leads to an extremely low probability of hazard arising from *E. coli* K–12 carrying recombinant DNA.

In response to the sixth and seventh comments given above that changes are being made "in the absence of data from a risk-assessment program," or upon insufficient data, I must note that the November 30 Decision Document/ Environmental Impact Assessment discussed in detail the substantial body of data available on the safety of *E. coli* K–12 and specifically dealt with the issue of risk-assessment under the discussions of the comments "Delay Any Change in the NIH Guidelines Pending Many More Risk-Assessment Experiments," and "Data Are Not Sufficient to Justify Exemption" as well as the alternative "Make No Change In The Guidelines Until Many More Risk-Assessment Experiments Are Completed." I continue to believe, as I wrote then, that the action is fully supported by the data.

III–B–2–d. Comments on the Time Taken To Promulgate the NIH Director's Decision on This Recommendation

Fourteen commentators, including one of the four RAC members who voted against the *"E. coli* K–12/P1 Recommendation" at the September 6, 1979, RAC meeting, wrote against delay, noting that the RAC's *"E. coli* K–12/P1 Recommendation" had been made in September 1979 but not yet promulgated. Comments included the following—"It is regrettable that these revisions have been delayed for further comment in view of the extensive period provided already for such comments and the extensive discussions by the Recombinant DNA Advisory Committee prior to its votes. I believe that the expense in terms of time taken from other fruitful activities of yourself and the many commentators on this issue was unnecessary and extremely wasteful."—"I am appalled at the interminable delays required before a recommendation of the RAC can be put into effect. The procedures required by the December 1978 guidelines are cumbersome enough without an additional layer of public comment, analysis, and justification added on. NIH and American biomedical scientists deserve better treatment and trust from their top health administrators."—"It is disheartening to find that, even after thorough consideration and approval by RAC, the *E. coli* K–12/P1 measure remains in administrative limbo."—"The unnecessarily long delays in implementing the new guidelines have adversely affected the morale of American scientists and hampered progress in this highly significant area of research and development."

On the other hand, one commentator wrote, "I once again congratulate you on the exemplary way in which revision of these guidelines is being continued while still making proposals available to the public for scrutiny before their final adoption."

I am firmly committed to the procedures of the NIH Guidelines. As pointed out above in Section II of this document, procedures for revising the Guidelines involve certain mandatory "delays" including publication of the proposed changes in the **Federal Register** for public comment, at least 30 days prior to a meeting of the RAC, and consideration of the proposed changes at a formal RAC meeting. For recommendations arising from three of the last four RAC meetings, there was no additional public comment period. For the recommendations made at the September 6–7, 1979, RAC meeting, however, I did issue my proposed decision for an additional 30-day period of public comment. It is my intention, generally, in the future, to rely, in formulating my final decision, on the comments received in the initial comment period, and on the recommendations of the RAC, without issuing a proposed decision for an additional period of public comment.

III–B–2–e. Ff Bacteriophages

Three letters discussed the use of Ff bacteriophages. One wrote, "Nor can I see why other *E. coli* K–12 host-vector systems, such as those employing Ff bacteriophages, are not included within the Section III–O reduction." Another wrote, "I certainly hope that this proposal will be extended to the Ff bacteriophages in the near future."

I discussed this in detail in my November 30, 1979, Decision Document/ Environmental Impact Assessment under the alternative "Include Ff Bacteriophages (Filamentous Single Strand Male Specific Bacteriophages such as M13 and fd) With Lambda or Lambdoid Bacteriophages To Be Permissible Under the *'E. coli* K–12/P1 Recommendation.' " There, I noted that I would "ask the RAC to consider the use of Ff bacteriophages again." At the December 1979 RAC meeting, a Working Group was appointed to consider this issue. They will report to the RAC at its next (March 1980) meeting. I will consider the recommendations of the RAC before taking action on inclusion of Ff bacteriophages within Section III–O.

III–B–2–f. Requirement for IBC Prior Review and Approval When There Is a Deliberate Attempt To Have the E. coli K–12 Efficiently Express a Gene Coding for a Eukaryotic Protein

One of the differences between the *"E. coli* K–12/P1 Recommendation" made by the RAC on September 6, 1979, and my proposed modification of this recommendation to become Section III–O of the November 30, 1979, proposed revised Guidelines was the addition of the text which states, "An exception, however, which does require prior review and approval by the IBC is any experiment in which there is a deliberate attempt to have the *E. coli* K–12 efficiently express any gene coding for a eukaryotic protein."

Four commentators wrote in opposition to this. One said that the requirement "is in my view superfluous, and is almost guaranteed to cause nuisance and confusion for investigators and IBC's. Many IBC's will understand this section to imply that they must require higher containment for such experiments, and for this the guidelines give no guidance or clarification. This requirement will expose many investigators to arbitrariness and unnecessary restrictions. . . . The Guidelines should clarify the intent of this requirement and should explicitly state that P1 containment is recommended. . . ." Two other commentators urged that this sentence be eliminated. One wrote, "Failing that

amendment, I must ask for a clarification of the intent of the sentence in question. As I read the relevant paragraph, these 'expression' experiments are understood to be appropriately carried out at the P1+EK1 level of containment. I am afraid that an alternate, presumably unintended, reading would be that each IBC is urged to set its own standards on these experiments. This policy, I am sure you would agree, would be disastrous."

On the other hand, three commentators wrote in favor.—"I agree with your decision to require that experiments in which there is a deliberate attempt to have expression of a eukaryotic gene be reviewed and approved by the local IBC prior to their being performed."

In response, I do not judge this requirement to be "superfluous." I discussed it in my November 30, 1979, Decision Document/Environmental Impact Assessment under the alternative "Treat Experiments Equally In Which There Is Or Is Not A Deliberate Attempt To Achieve Gene Expression." There, I concluded the discussion on this issue by stating, "Therefore, experiments in which there is a deliberate attempt to achieve gene expression continue to merit special attention. . . . This will allow the IBC to judge whether it wishes to require any added restrictions to be placed on the experiment, and to remain fully informed of its progress."

In response to the request that the Guidelines "should explicitly state that P1 containment is recommended," I note that the Guidelines do explicitly state in Section III–O that ". . . experiments using *E. coli* K–12 shall use P1 physical containment. . . ." including those "in which there is a deliberate attempt to have the *E. coli* K–12 efficiently express any gene coding for a eukaryotic protein. It is not NIH's intention that the IBC must require higher containment for such experiments.

One commentator suggested a rewording of this sentence as follows—"An exception, however, which does require prior review and approval by the IBC is any experiment in which there is a deliberate attempt to have the *E. coli* K–12 efficiently express as a protein product the information carried in any gene derived either from a eukaryotic organism or from any virus or viroid which infects a eukaryotic organism." I will have this suggestion published for at least 30 days public comment, and will ask the RAC to consider it at its next (March 1980) meeting before I take action on it.

III–B–2–g. Use of Poorly Mobilizable Plasmids

One commentator suggested that experiments described in Section III–O of the Guidelines which specify that "the host shall not contain conjugation-proficient plasmids" add an additional safety feature by the use of "a poorly mobilizable plasmid. By that I mean one that is mobilizable at frequencies of $<10^{-5}$ by a derepressed conjugative plasmid." I will have this suggestion published for at least 30 days public comment and will ask that it be considered first by the RAC Subcommittee on Host-Plasmid Vector Systems, and then by the full RAC at its March 1980 meeting, before I take action on it.

III–B–2–h. Transfer of Clones to Other Laboratories

One correspondent discussed "the requirement that clones subject to the guidelines can be transferred to other laboratories only after the recipient submits an approved MUA to the supplying laboratory" and questioned whether this should apply to clones described in Section III–O of the Guidelines.

Detailed instructions on the administration of the NIH Guidelines are contained in the "Administrative Practices Supplement to the NIH Guidelines for Research Involving Recombinant DNA Molecules" (APS). Currently, a Memorandum of Understanding and Agreement (MUA) is required to be submitted to NIH for each NIH-funded recombinant DNA project subject to the Guidelines. As described in the APS, the MUA must contain a statement "agreeing to abide by the provisions of the NIH Guidelines and the requirements of this Supplement concerning shipment and transfer of recombinant DNA materials." The revised NIH Guidelines, issued today, specify that for experiments described in Section III–O, "no Memorandum of Understanding and Agreement (MUA) . . . need be submitted . . ." NIH will soon issue a revised version of the APS taking into account the changes in the Guidelines promulgated today. At that time, requirements concerning shipment of clones described under Section III–O of the Guidelines will be described.

III–B–3. Comments on the Proposed Revised Guidelines Other Than Section III–O

Only three comments were received dealing with a "major action" recommended at the September 6–7, 1979, RAC meeting, other than the "*E. coli* K–12/P1 Recommendation." These three requested that the Proposed Supplement (Part VI) on Voluntary Compliance not be added to the Guidelines. The reason given by one commentator was that it may "lead to unnecessary and wasteful legislative attempts." The other two commentators, on the other hand, specifically called for mandatory compliance.

In my November 30 Decision Document, I reviewed the history of this proposed supplement in detail including endorsement of it by the Federal Interagency Committee on Recombinant DNA Research and by the RAC. In accord with the analysis in that document, I accept the recommendation of these two committees to add Part VI to the Guidelines.

III–B–4. Comments on the Guidelines Other Than Changes Recommended by the RAC

The Decision Document/Environmental Impact Assessment/Proposed Revised Guidelines, as published for public comment on November 30, were based upon changes in the Guidelines recommended by the RAC at its September 6–7, 1979, meeting. During the comment period, five letters were received proposing additional changes in the Guidelines totally unrelated to the RAC recommendations. One commentator requested exemption from the Guidelines of "return to host of origin" type experiments. One commentator requested that the Institutional Biosafety Committee members not affiliated with the institution "shall be appointed by the governing body of the community in which the institution is situated." Two commentators submitted a proposed addition to Appendix B of the Guidelines to deal with plant pathogens. One commentator requested elimination of Prohibition I–D–3, and a revision of Sublist A of Appendix A to the Guidelines.

I will have the proposals mentioned above under III–B–4 published for at least 30 days public comment, and will ask the RAC to consider them at its next (March 1980) meeting before I take action on them.

III–C. Decision of the NIH Director on Promulgation of Revised Guidelines

Based on my analysis of the comments received during this comment period, I am today promulgating revised NIH Guidelines for Research Involving Recombinant DNA Molecules. They differ from the proposed revised Guidelines as published in the **Federal Register** on November 30, 1979, by the incorporation of the additional changes which were recommended by the RAC at its December 6–7, 1979, meeting, and which were promulgated in the **Federal Register** on January 7, 1980 (45 FR 3552).

Dated: January 23, 1980.

Donald S. Fredrickson,

Director, National Institutes of Health.

[FR Doc. 80-2821 Filed 1-28-80; 8:45 am]

BILLING CODE 4110-08-M

federal register

Tuesday
January 29, 1980

Part VI

Department of Health, Education, and Welfare

National Institutes of Health

Guidelines for Research Involving Recombinant DNA Molecules

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

National Institutes of Health

Guidelines for Research Involving Recombinant DNA Molecules

January 1980.

Contents

I. Scope of the Guidelines

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

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

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

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

I–D–1. Formation of recombinant DNAs derived from the pathogenic organisms classified (*1*) as Class 3, 4, or 5 (*2*) or from cells known (*2A*) to be infected with such agents, regardless of the host-vector system used.

I–D–2. Deliberate formation of recombinant DNAs containing genes for the biosynthesis of toxins potent for vertebrates (*2A*) (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. (*2A*)

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

I–D–5. Deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire it naturally, if such acquisition could compromise the use of a drug to control disease agents in human or veterinary medicine or agriculture. (*2A*)

I–D–6. Large-scale experiments (e.g., more than 10 liters of culture) with organisms containing recombinant DNAs, unless the recombinant DNAs are rigorously characterized and the absence of harmful sequences established (*3*). (See Section IV–E–1–b–(3)–(d).)

We differentiate between small- and large-scale experiments with organisms containing recombinant DNAs because the probability of escape from containment barriers normally increases with increasing scale.

Experiments in Categories I–D–1 to I–D–6 may be excepted (*4*) from the prohibitions (and will at that time be assigned appropriate levels of physical and biological containment) provided that these experiments are expressly approved by the Director, NIH, with advice of the Recombinant DNA Advisory Committee after appropriate notice and opportunity for public

comment. (See Section IV–E–1–b–(1)–(e).)

Experiments in Categories I–D–1, I–D–2, I–D–3, I–D–5, and experiments involving "wild type" host-vector systems are excepted from the prohibitions, provided that these experiments are designed for risk-assessment purposes and are conducted within the NIH high-containment facilities located in Building 41-T on the Bethesda campus and in Building 550 located at the Frederick Cancer Research Center. The selection of laboratory practices and containment equipment for such experiments shall be approved by ORDA following consultation with the RAC Risk-Assessment Subcommittee and the NIH Biosafety Committee. ORDA shall inform RAC members of the proposed risk-assessment projects at the same time it seeks consultation from the RAC Risk-Assessment Subcommittee and the NIH Biosafety Committee. If a major biohazard is determined, the clones will be destroyed after the completion of the experiment rather than retaining them in the high containment facility. Other clones that are non-hazardous or not of major hazard will be retained in the high containment.

I–E. *Exemptions.* It must be emphasized that the following exemptions(*4*) are not meant to apply to experiments described in the Sections I–D–1 to I–D–5 as being prohibited.

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

I–E–1. Those that are not in organisms or viruses.(*5*)

I–E–2. Those that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent.

I–E–3. Those that consist entirely of DNA from a prokaryotic host, including its indigenous plasmids or viruses, when propagated only in that host (or closely related strain of the same species) or when transferred to another host by well established physiological means; also those that consist entirely of DNA from a eukaryotic host, including its chloroplasts, mitochondria, or plasmids (but excluding viruses), when propagated only in that host (or a closely related strain of the same species).

I–E–4. Certain specified recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the Director, NIH, with advice of the Recombinant DNA Advisory Committee, after appropriate notice and opportunity for public comment. (See Section IV–E–1–b–(1)–(d).) Certain classes are exempt as of publication of these Revised Guidelines. The list is in Appendix A. An updated list may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20205.

I–E–5. Other classes of recombinant DNA molecules, if the Director, NIH, with advice of the Recombinant DNA Advisory Committee, after appropriate notice and opportunity for public comment, finds that they do not present a significant risk to health or the environment. (See Section IV–E–1–b–(1)–(d).) Certain classes are exempt as of publication of these Revised Guidelines. The list is in Appendix C. An updated list may be obtained from the Office of Recombinant DNA Activities; National Institutes of Health, Bethesda, Maryland 20205.

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

II. Containment

Effective biological safety programs have been operative in a variety of laboratories for many years. Considerable information therefore already exists for the design of physical containment facilities and the selection of laboratory procedures applicable to organisms carrying recombinant DNAs.(*6–19*) The existing programs rely upon mechanisms that, for convenience, can be divided into two categories: (i) a set of standard practices that are generally used in microbiological laboratories, and (ii) special procedures, equipment, and laboratory installations that provide physical barriers which are applied in varying degrees according to the estimated biohazard.

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

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

In constructing these Guidelines, it was necessary to define boundary conditions for the different levels of physical and biological containment and for the classes of experiments to which they apply. We recognize that these definitions do not take into account all existing and anticipated information on special procedures that will allow particular experiments to be carried out under different conditions 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 DNAs must receive adequate instruction. (See Sections IV–D–1–g, IV–D–5–d and IV–D–8–b.). This shall as a minimum include instructions in aseptic techniques and in the biology of the organisms used in the experiments, so that the potential biohazards can be understood and appreciated.

Any research group working with agents with a known or potential biohazard shall have an emergency plan which describes the procedures to be followed if an accident contaminates personnel or the environment. The principal investigator must ensure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan. (See Sections IV–D–5–e and IV–D–3–d.) If a research group is working with a known pathogen where there is an effective vaccine it should be made available to all workers. Where serological monitoring is clearly appropriate it shall be provided. (See Sections IV–D–1–h and IV–D–8–c.)

II–B. *Physical Containment Levels.* The objective of physical containment is to confine organisms containing recombinant DNA molecules, and thus

to reduce the potential for exposure of the labortory worker, persons outside of the laboratory, and the environment to organisms containing recombinant DNA molecules. Physical containment is achieved through the use of laboratory practices, containment equipment, and special laboratory design. Emphasis is placed on primary means of physical containment which are provided by laboratory practices and containment equipment. Special laboratory design provides a secondary means of protection against the accidental release of organisms outside the laboratory or to the environment. Special laboratory design is used primarily in facilities in which experiments of moderate to high potential 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 approaches 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 selections of alternative methods of primary containment is dependent, however, on the level of biological containment provided by the host-vector system used in the experiment. Consideration will also be given by the Director, NIH, with the advice of the Recombinant DNA Advisory Committee to other combinations which achieve an equivalent level of containment. [See Section IV–E–1–b–(2)–(b).] Additional material on physical containment for plant host-vector systems is found in Sections III–C–3 and III–C–4.

II–B–1. *P1 Level.*

II–B–1–a. *Laboratory Practices.*

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

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

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

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

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

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

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

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

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

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

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

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

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

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

II–B–2. *P2 Level.*

II–B–2–a. *Laboratory Practices.*

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

II–B–2–b. *Containment Equipment.* Biological safety cabinets [20] shall be used to contain aerosol-producing equipment, such as blenders, lyophilizers, sonicators, and centrifuges, when used to process organisms containing recombinant DNA molecules.

except where equipment design provides for containment of the potential aerosol. For example, a centrifuge may be operated in the open if a sealed head or safety centrifuge cups are used.

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

II–B–3. *P3 Level.*

II–B–3–a. *Laboratory Practices.*

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

II–B–3–b. *Containment Equipment.*

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

II–B–3–b–(2). Laboratory animals held in a P3 area shall be housed in partial-containment caging systems, such as Horsfall units (*19A*), open cages placed in ventilated enclosures, solid-wall and solid-bottom cages covered by filter bonnets, or solid-wall and solid-bottom cages placed on holding racks equipped with ultraviolet radiation lamps and reflectors. (Note: Conventional caging systems may be used, provided that all personnel wear appropriate personal protective devices. These shall include, at a minimum, wrap-around gowns, head covers, gloves, shoe covers, and respirators. All personnel shall shower on exit from areas where these devices are required.

II–B–3–b–(3). *Alternative Selection of Containment Equipment.* Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified in Part III can be conducted in the P3 laboratory using containment equipment specified for the P2 level of physical containment. Experimental procedures involving a host-vector system that provides a one-step lower level of biological containment than that specified in Part III can be conducted in the P3 laboratory using containment equipment specified for the P4 level of physical containment. Alternative combinations 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 by a controlled access area from areas that are open to unrestricted traffic flow. A controlled access area is an anteroom, a change room, an air lock or any other double-door arrangement that separates the laboratory from areas open to unrestricted traffic flow.

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

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

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

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

II–B–4. *P4 Level.*

II–B–4–a. *Laboratory Practices.*

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

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

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

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

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

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

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

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

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

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

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

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

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

II–B–4–a–(14). Street clothing shall be removed in the outer side of the clothing-change area and kept there. Complete laboratory clothing, including undergarments, head cover, shoes, and

either pants and shirts or jumpsuits, shall be used by all persons who enter the P4 facility. Upon exit, personnel shall store this clothing in lockers provided for this purpose or discard it into collection hampers before entering the shower area.

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

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

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

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

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

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

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

II–B–4–b. *Containment Equipment.*

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

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

II–B–4–b–(3). *Alternative Selection of Containment Equipment.* Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment 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.
** In this case gloves shall be worn, in addition to the clothing requirements specified in II–B–4–a–(14).

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

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

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

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

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

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

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

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

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

II–B–4–c–(9). All liquid effluents from the P4 facility shall be collected and decontaminated before disposal. Liquid effluents from biological safety cabinets and laboratory sinks shall be sterilized by heat. Liquid effluents from the shower and hand washing facilities may be inactivated by chemical treatment.

HEPA filters shall be installed in all vents from effluent drains.

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

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

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

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

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

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

II–C. *Shipment.* Recombinant DNA molecules contained in an organism or virus shall be shipped only as an etiologic agent under requirements of the U.S. Public Health Service and the U.S. Department of Transportation (Section 72.25, Part 72, Title 42, and Sections 173.386–.388, Part 173, Title 49, U.S. Code of Federal Regulations) as specified below:

II–C–1. Recombinant DNA molecules contained in an organism or virus requiring P1, P2, or P3 physical containment, when offered for transportation or transported, are subject to all requirements of Section 72.25(c)(1)–(5), Part 72, Title 42 CFR, and § 173.386–.388, Part 173, Title 49 CFR.

II–C–2. Recombinant DNA molecules contained in an organism or virus requiring P4 physical containment, when offered for transportation or transported, are subject to the requirements listed above under II–C–1 and are also subject to Section 72.25(c)(6), Part 72, Title 42 CFR.

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

II–D. *Biological Containment.*

II–D–1. *Levels of Biological Containment.* In consideration of biological containment, the vector (plasmid, organelle, or virus) for the recombinant DNA and the host (bacterial, plant, or animal cell) in which the vector is propagated in the laboratory will be considered together. Any combination of vector and host which is to provide biological containment must be chosen or constructed so that the following types of "escape" are minimized: (i) survival of the vector in its host outside the laboratory and (ii) transmission of the vector from the propagation host to other nonlaboratory hosts.

The following levels of biological containment (HV, or *H*ost-*V*ector, systems) for prokaryotes will be established; specific criteria will depend on the organisms to be used. Eukaryotic host-vector systems are considered in Part III.

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

II–D–1–a–(1). *EK1.* The host is always *E. coli* K–12 or a derivative thereof, and the vectors include nonconjugative plasmids (e.g., pSC101, Co1E1, or derivatives thereof (*21–27*) and variants of bacteriophage, such as lambda (28–33). The *E. coli* K–12 host shall not contain conjugation-proficient plasmids, whether autonomous or integrated, or generalized transducing phages, except as specified in Section III–0.

II–D–1–a–(2). *Other Prokaryotes.* Hosts and vectors shall be, at a minimum, comparable in containment to *E. coli* K–12 with a non conjugative 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 (in the inserted or plasmid form) in the laboratory host used for phage propagation or (ii) by surviving in natural environments and transferring a cloned DNA fragment to other hosts (or their resident prophages).

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

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

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

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

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

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

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

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

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

When new host-vector systems are certified, notice of the certification will be sent by ORDA 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.

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

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

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

II-D-2-b-(1). *HV1 Systems Other than E. Coli K–12.* The following types of data shall be submitted, modified as appropriate for the particular system under consideration: (i) A description of the organism and vector; the strain's natural habitat and growth requirements; its physiological properties, particularly those related to its reproduction and survival and the mechanisms by which it exchanges genetic information; the range of organisms with which this organism normally exchanges genetic information and what sort of information is exchanged; and any relevant information on its pathogenicity or toxicity. (ii) A description of the history of the particular strains and vectors to be used, including data on any mutations which render this organism less able to survive or transmit genetic information. (iii) A general description of the range of experiments contemplated, with emphasis on the need for developing such an HV1 system.

II-D-2-b-(2). *HV2 Systems.* Investigators planning to request HV2 certification for host-vector systems can obtain instructions from ORDA concerning data to be submitted [33A, 33B]. In general, the following types of data are required: (i) Description of construction steps, with indication of source, properties, and manner of introduction of genetic traits. (ii) Quantitative data on the stability of genetic traits that contribute to the containment of the system. (iii) Data on the survival of the host-vector system under nonpermissive laboratory conditions designed to represent the relevant natural environment. (iv) Data on transmissibility of the vector and/or cloned DNA fragment under both permissive and nonpermissive conditions. (v) Data on all other properties of the system which affect containment and utility, including information on yields of phage or plasmid molecules, ease of DNA isolation, and ease of transfection or transformation. (vi) In some cases, the investigator may be asked to submit data on survival and vector transmissibility from experiments in which the host-vector is fed to laboratory animals (e.g., rodents). Such *in vivo* data may be required to confirm the validity of predicting *in vivo* survival on the basis of *in vitro* experiments.

Data must be submitted in writing to ORDA. Ten to twelve weeks are normally required for review and circulation of the data prior to the meeting at which such data can be considered by the 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 bacteriophage λ 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 environments.

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

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

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

reported properties or any problems in the safe use of the system.

NIH may also distribute certified HV1 host-vector systems.

III. Containment Guidelines for Covered Experiments

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

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

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

III–O. *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.

Some experiments using *E. coli* K–12 host-vector systems are prohibited (see Section I–D).

Some experiments using *E. coli* K–12 host-vector systems are exempt from the Guidelines (see Section I–E).

Other experiments using *E. coli* K–12 shall use P1 physical containment and, except as specified in the last paragraph of this section, an EK1 host-vector system (i.e. (a) the host shall not contain conjugation-proficient plasmids or generalized transducing phages, and (b) lambda or lambdoid bacteriophages or non-conjugative plasmids shall be used as vectors). For these experiments no Memorandum of Understanding and Agreement (MUA) as described in Section IV–D–1–c need be submitted, nor is any registration with NIH necessary. However, for these experiments, prior to their initiation, investigators must submit to their Institutional Biosafety Committee (IBC) a registration document that contains a description of (a) the source(s) of DNA, (b) the nature of the inserted DNA sequences, and (c) the hosts and vectors to be used. This registration document must be dated and signed by the investigator and filed only with the local IBC. The IBC shall review all such proposals but such review is not required prior to initiation of experiments. An exception, however, which does require prior review and approval by the IBC is any experiment in which there is a deliberate attempt to have the *E. coli* K–12 efficiently express any gene coding for a eukaryotic protein.

Experiments involving the insertion into *E. coli* K–12 of DNA from prokaryotes that exchange genetic information with *E. coli* by known physiological processes will be exempted from these Guidelines if they appear on the "list of exchangers" set forth in Appendix A (see Section I–E–4).

For those not on the Appendix A list but which exchange genetic information [35] with *E. coli*, experiments may be performed with any *E. coli* K–12 vector (e.g. conjugative plasmid). When a non-conjugative vector is used, the *E. coli* K–12 host may contain conjugation-proficient plasmids, either autonomous or integrated, or generalized transducing phages.

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

It has been necessary, throughout this section, to use words and terms marked with footnote reference numbers. The footnotes (Part V) define more fully what the terms denote.

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

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

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

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

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

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

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

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

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

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

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

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

III–A–2–a–(1)–(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 HV1 host-vector shall be used for DNA recombinants produced with (i) the whole viral genome, (ii) subgenomic DNA segments, or (iii) purified cDNA copies of viral mRNA.(*37*)

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

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

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

III–A–2–a–(1)–(a)–(*2*)–(*c*). P2 physical containment + an HV1 host and a vector certified for use in an HV2 system, or P3 + HV1, shall be used for DNA recombinants derived from purified cDNA copies of viral mRNA.(*37*)

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

III–A–2–a–(1)–(a)–(*3*)–(*a*). P1 physical containment + an HV1 host-subgenomic DNA(*38*) segments or (ii) purified cDNA copies of viral mRNA.(*37*)

III–A–2–a–(1)–(a)–(*3*)–(*b*). P1 physical containment + an HV1 host and a vector certified for use in an HV2 system shall be used for DNA recombinants produced with the whole viral genome or with subgenomic segments that have not been purified to the extent required in footnote 38.

III–A–2–a–(1)–(a)–(b). *Transforming Viruses.(37A)*

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

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

III–A–2–a–(1)–(b)–(*1*)–(*b*). P2 physical containment + an HV1 host and a vector certified for use in an HV2 system, or P3 + HV1, shall be used for (i) DNA recombinants produced with purified subgenomic DNA segments containing an entire transforming gene(*38*) or (ii) purified cDNA copies of viral mRNA.(*37*)

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

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

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

III–A–2–a–(1)–(b)–(*2*)–(*b*). P2 physical containment + an HV1 host and a vector certified for use in an HV2 system, or P3 + HV1, shall be used for (i) DNA recombinants produced with the whole viral genome, (ii) subgenomic DNA segments containing an entire transforming gene, (iii) purified cDNA copies of viral mRNA, (*37*) or (iv) subgenomic segments that have not been purified to the extent required in footnote 38.

III–A–2–a–(2). *DNA Transcripts of RNA Viruses.*

III–A–2–a–(2)–(a). *Retroviruses.*

III–A–2–a–(2)–(a)–(*1*). *Gibbon Ape, Woolly Monkey, Feline Leukemia and Feline Sarcoma Viruses.(39).*

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

III–A–2–a–(2)–)a)–(*1*)–(*b*). P2 physical containment + an HV1 host and a vector certified for use in an HV2 system, or P3 + HV1, shall be used for DNA recombinants produced with purified subgenomic DNA segments (*38*) containing an entire transforming gene.

III–A–2–a–(2)–(a)–(*1*)–(*c*). P2 physical containment + an HV2 host-vector, or P3 + HV1, shall be used for DNA recombinants produced with (i) the whole viral genome, (ii) purified cDNA copies of viral mRNA,(*37*) or (iii) subgenomic segments that have not been purified to the extent required in footnote 38.

III–A–2–a–(2)–(a)–(*2*). *Other Members of the Family Retroviridiae.(36)*

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

III–A–2–a–(2)–(a)–(*2*)–(*b*). P2 physical containment + an HV1 host and a vector certified for use in an HV2 system, or P3 + HV1, shall be used for DNA recombinants produced with (i) subgenomic DNA segments containing an entire transforming gene, (ii) the whole viral genome, or (iii) purified cDNA copies of viral mRNA.(*37*) or (iv) subgenomic segments that have not been purified to the extent required in footnote 38.

III–A–2–a–(2)–(b). *Negative Strand RNA Viruses.* P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with (i) cDNA copies of the whole genome, (ii) subgenomic cDNA segments, or (iii) purified cDNA copies of viral mRNA.(*37*)

III–A–2–a–(2)–(c). *Plus-Strand RNA Viruses.*

III–A–2–a–(2)–(c)–(*1*). *Types 1 and 2 Sabin Poliovirus Vaccine Strains and Strain 17D (Theiler) of Yellow Fever Virus.* P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with (i) cDNA copies of the whole viral genome, (ii) subgenomic cDNA segments, or (iii) purified cDNA copies of viral mRNA.(*37*)

III–A–2–a–(2)–(c)–(*2*). *Other Plus-Strand RNA Viruses Belonging to Presently Classified Viral Families.(36)*

BILLING CODE 4110-08-M

Table III

Recommended Containment for Cloning of Viral DNA or cDNA in Certain HV1 and HV2 Systems Specified in Appendix D
(See text for full details)

Virus class	Type of viral DNA segment to be cloned				
	Subgenomic[38]		Genomic*		cDNA from viral mRNA[37]
	Nontransforming segment	Segment containing an entire transforming gene	Nonsegmented genome	Segmented genome	
DNA					
Nontransforming viruses					
AAV, MVM, Mouse Adeno (Strain FL)	P1 + HV1		P1 + HV1		P1 + HV1
Plant Viruses	P1 + HV1		P1 + HV1		P1 + HV1
Hepatitis B	P1 + HV1[38]		P2 + HV2 or P3 + HV1		P2 + HV1CV[40] or P3 + HV1
Other	P1 + HV1[38]		P1 + HV1CV[40]		P1 + HV1
Transforming Viruses					
Herpes Saimiri, H. Ateles and EBV[39]	P1 + HV1[38]	P2 + HV1CV[40] or P3 + HV1[38]	P2 + HV2 or P3 + HV1		P2 + HV1CV[40] or P3 + HV1
Other	P1 + HV1[38]	P2 + HV1CV[40] or P3 + HV1	P2 + HV1CV[40] or P3 + HV1		P2 + HV1CV[40] or P3 + HV1
RNA					
Retroviruses					
Gibbon Ape, Woolly Monkey FeLV and FeSV[39]	P1 + HV1[38]	P2 + HV1CV[40] or P3 + HV1[38]	P2 + HV2 or P3 + HV1		P2 + HV2 or P3 + HV1
Other	P1 + HV1[38]	P2 + HV1CV[40] or P3 + HV1	P2 + HV1CV[40] or P3 + HV1		P2 + HV1CV[40] or P3 + HV1

* See exception given at asterisk at end of Appendix D

Table III, continued

Recommended Containment for Cloning of Viral DNA or cDNA in Certain HV1 and HV2 Systems Specified in Appendix D
(See text for full details)

Virus class	Type of viral DNA segment to be cloned				
	Subgenomic[38]		Genomic*		cDNA from viral mRNA[37]
	Nontransforming segment	Segment containing an entire transforming gene	Nonsegmented genome	Segmented genome	
Negative-Strand RNA	P1 + HV1		P1 + HV1	P1 + HV1	P1 + HV1
Plus-Strand RNA					
Types 1 and 2 Sabin Polio, 17D Yellow Fever Vaccine Strains	P1 + HV1		P1 + HV1		P1 + HV1
Other	P1 + HV1[38]		P2 + HV1CV[40] or P3 + HV1		P2 + HV1CV[40] or P3 + HV1
Double-Stranded RNA	P1 + HV1			P1 + HV1	P1 + HV1
Plant Viruses + Viroids	P1 + HV1		P1 + HV1	P1 + HV1	P1 + HV1
Intracellular Viral DNA	See text	See text	See text		

* See exception given at asterisk at end of Appendix D

BILLING CODE 4110-08-C

III–A–2–a–(2)–(c)–(*2*)–(*a*). P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with purified subgenomic cDNA segments. (*38*)

III–A–2–a–(2)–(c)–(*2*)–(*b*). P2 physical containment + an HV1 host and a vector certified for use in an HV2 system, or P3 + HV1, 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–a–A–(2)–(d). *Double-Stranded Segmented RNA Viruses.* P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with (i) mixtures of subgenomic cDNA segments, (ii) a specific subgenomic cDNA segment, or (iii) purified cDNA copies of viral mRNA. (*37*)

III–A–2–a–(2)–(e). *RNA Plant Viruses and Plant Viroids.* P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with (i) cDNA copies of the whole viral genone, (ii) subgenomic cDNA segments, or (iii) purified cDNA copies of viral mRNA. (*37*)

III–A–2–a–(3). *Intracellular Viral DNA.* Physical and biological containment specified for shotgun experiments with eukaryotic cellular DNA [see Section III–A–(1)–(a)] shall be used for DNA recombinants produced with integrated viral DNA or viral genomes present in infected cells.

III–A–2–b. *Eukaryotic Organelle DNAs.* P2 physical containment + an HV1 host-vector, or P1 + HV2, for mitochondrial or chloroplast DNA from eukaryotes when the organelle DNA has been obtained from isolated organelles. Otherwise, the conditions given for shotgun experiments apply.

III–A–2–c. *Prokaryotic Plasmid and Phage DNAs.* The containment levels required for shotgun experiments with DNA from prokaryotes apply to their plasmids or phages (See Section III–A–1–b).

III–A–3. *Lowering of Containment Levels for Characterized or Purified DNA Preparations and Clones.* Many of the risks which might conceivably arise from some types of recombinant DNA experiments, particularly shotgun experiments, would result from the inadvertant cloning of a harmful sequence. Therefore, in cases where the risk of inadvertently cloning the "wrong" DNA is reduced by prior enrichment for the desired piece, or in which a clone made from a random assortment of DNAs has been purified and the absence of harmful sequences established, the containment conditions for further work may be reduced. The following section outlines the mechanisms for such reductions.

III–A–3–a. *Purified DNA Other than Plasmids, Bacteriophages, and Other Viruses.* The formation of DNA recombinants from cellular DNAs that have been purified (*41*) and in which the absence of harmful sequences has been established (*3*) can be carried out under lower containment conditions than used for the corresponding shotgun experiment. (*42*) The containment may be decreased one step in physical containment (P4→P3; P3→P2; P2→P1) while maintaining the biological containment specified for the shotgun experiment, or one step in biological containment (HV3→HV2; HV2→HV1) while maintaining the specified physical containment. The institutional biosafety committee (IBC) must review such a reduction and the approval of the IBC and of the NIH must be secured before such a reduction may be put into effect.

III–A–3–b. *Characterized Clones of DNA Recombinants.* When a cloned DNA recombinant has been rigorously characterized and the absence of harmful sequences has been established (*3*), experiments involving this recombinant DNA may be carried out under lower containment conditions, with the prior approval of the IBC and of NIH.

III–B. *Experiments with Prokaryotic Host-Vectors Other Than E. coli K–12*

III–B–1. *HV1 and HV2 Systems.* Certain certified HV1 and HV2 host-vector systems appear in Appendix D. The containment levels for these systems are given in the subsections of Section III–A. Other systems in the future may be certified as HV1 and HV2. At the time of certification, the classification of containment levels for experiments using them will be assigned by NIH.

III–B–2. *Return of DNA Segments to Prokaryotic Non-HV1 Host of Origin.* Certain experiments involving those prokaryotes that exchange genetic information with *E. coli* by known physiological processes will be exempt from these Guidelines if they appear on the "list of exchangers" set forth in Appendix A (see Section I–E–4). For a prokaryote which can exchange genetic information (*35*) with *E. coli* under laboratory conditions but which is not on the list (Host A), the following type of experiment may be carried out under P1 conditions without Host A having been approved as an HV1 host: DNA from Host A may be inserted into a vector and propagated in *E. coli* K–12 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 and propagated in *E. coli* K–12 under P1 conditions. Subsequently, the recombinant DNA may be returned to Host B and propagated in Host B under P1 conditions. (*43*)

III–B–3. *Non-HV1 Systems.* Containment levels for other classes of experiments involving non-HV1 systems may be approved by the Director, NIH. (See Sections IV–E–1–b–(1)–(b), IV–E–1–b–(2)–(c), and IV–E–1–b–(3)–(b).)

In those cases where genetic exchange has not been demonstrated between two bacterial species A and B, neither of which is known to be pathogenic for man, animals, or plants, recombinant DNA experiments involving only A and B can be conducted under P3 containment. (*2A*)

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 whole polyoma virus genomes, with appropriate helper, if necessary, can be used in P2 conditions to propagate DNA sequences:

III–C–1–a–(1)–(a)–(*1*). from bacteria of class 1 or class 2 (*1*) or their phages or plasmids, except for those that produce potent polypeptide toxins; (*34*)

III–C–1–a–(1)–(a)–(*2*). from mice;

III–C–1–a–(1)–(a)–(*3*). from eukaryotic organisms that do not produce potent polypeptide toxins, (*34*) provided that the DNA segment is >99% pure.

III–C–1–a–(1)–(b). Defective polyoma genomes, with appropriate helper, if necessary, can be used in P2 conditions for shotgun experiments to propagate DNA sequences from eukaryotic organisms that do not produce potent polypeptide toxins. (*34*)

III–C–1–a–(1)–(c). Whole virus genomes with appropriate helper, if necessary, can be used in P3 conditions for shotgun experiments to propagate DNA sequences from eukaryotic organisms that do not produce potent polypeptide toxins. (*34*)

III–C–1–a–(1)–(d). Experiments involving the use of defective polyoma virus genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by NIH on a case-by-case basis (*45*) and will be conducted under

the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

III–C–1–a–(2). *Nonproductive Virus-Cell Interactions.* Defective or whole polyoma virus genomes can be used as vectors in P2 conditions when production of viral particles cannot occur (e.g., transformation of nonpermissive cells or propagation of an unconditionally defective recombinant genome in the absence of helper), provided the inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis (*45*) and will be conducted under the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

III–C–1–b. *Simian Virus 40.*

III–C–1–b–(1). *Productive Virus-Cell Interactions.*

III–C–1–b–(1)–(a). SV40 DNA, rendered unconditionally defective by a deletion in an essential gene, with appropriate helper, can be used in P2 conditions to propagate DNA sequences from:

III–C–1–b–(1)–(a)–(*1*). bacteria of Class 1 or Class 2, (*1*) or their phages or plasmids, except for those that produce potent polypeptide toxins; (*34*)

III–C–1–b–(1)–(a)–(*2*). Unifected 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 a appropriate helper, can be used in P3 conditions to propagate DNA sequences from eukaryotic organisms that do not produce potent polypeptide toxins (*34*) (shotgun experiments or purified DNA).

III–C–1–b–(1)–(c). Experiments involving the use of defective SV40 genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by NIH on a case-by-case basis[45] and will be conducted under the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

III–C–1–b–(2). *Nonproductive Virus-Cell Interactions.* Defective or whole SV40 genomes can be used as vectors in P2 conditions when production of viral particles cannot occur (e.g., transformation of nonpermissive cells or propagation of an unconditionally defective recombinant genome in the absence of helper), provided the inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis [45] and will be conducted under the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

III–C–1–c. *Human Adenoviruses 2 and 5.*

III–C–1–c–(1). *Productive Virus–Cell Interactions.*

III–C–1–c–(1)–(a). Human adenoviruses 2 and 5, rendered unconditionally defective by deletion of at least two essential genes, with appropriate helper, can be used in P3 conditions to propagate DNA sequences from:

III–C–1–c–(1)–(a)–(*1*). bacteria of Class 1 or Class 2 (*1*) or their phages or plasmids except for those that produce potent polypeptide toxins; [34]

III–C–1–c–(1)–(a)–(*2*). eukaryotic organisms that do not produce potent polypeptide toxins[34] (shotgun experiments or purified DNA).

III–C–1–(c)–(1)–(b). Experiments involving the use of unconditionally defective human adenovirus 2 and 5 genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by NIH on a case-by-case basis [45] and will be conducted under the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

III–C–1–c–(2). *Nonproductive Virus-Cell Interactions.* Defective or whole human adenovirus 2 and 5 genomes can be used as vectors in P2 conditions when production of viral particles cannot occur (e.g., transformation of nonpermissive cells or propagation of an unconditionally defective recombinant genome in the absence of helper), provided the inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis (45) and will be conducted under the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

III–C–1–d. *Murine Adenovirus Strain FL.*

III–C–1–d–(1). *Productive Virus-Cell Interactions.*

III–C–1–d–(1)–(a). Unconditionally defective murine adenovirus strain FL genomes, with appropriate helper, can be used in P2 conditions to propagate DNA sequences from:

III–C–1–d–(1)–(a)–(*1*). bacteria of Class 1 or Class 2 (*1*) or their phages or plasmids except for those that produce potent polypeptide toxins;[34]

III–C–1–d–(1)–(a)–(*2*). eukaryotic organisms that do not produce potent polypeptide toxins[34] (shotgun experiments or purified DNA).

III–C–1–d–(1)–(b). Experiments involving the use of whole murine adenovirus strain FL genomes to propagate DNA sequences from prokaryotic or eukaryotic organisms will be evaluated by NIH on a case-by-case basis[45] and will be conducted under the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

III–C–1–d–(1)–(c). Experiments involving the use of unconditionally defective murine adenorvirus strain FL genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by NIH on a case-by-case basis (*45*) and will be conducted under the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

III–C–1–d–(2). *Nonproductive Virus-Cell Interactions.* Defective or whole murine adenovirus strain FL genomes can be used as vectors in P2 conditions when production of viral particles cannot occur (e.g., transformation of nonpermissive cells or propagation of an unconditionally.defective recombinant genome in the absence of helper), provided the inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis (*45*) and will be conducted under the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

III–C–1–e. *All Other Potential Viral Vectors.*

III–C–1–e–(1). Experiments involving recombinant DNA molecules containing viral DNA segments consisting of 25% 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] and will be conducted under the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

III–C–1–e–(1)–(b). under physical and biological containment conditions to be determined by NIH (*45*) when a viral helper will be used to propagate DNA sequences from prokaryotic or eukaryotic organisms. (See Section IV–E–1–b–(3)–(c).)

III–C–1–e–(2). Experiments involving the use of other whole or defective virus genomes to propagate DNA sequences from prokaryotic or eukaryotic organisms (and viruses), or as vectors to transform nonpermissive cells, will be evaluated by NIH on a case-by-case basis (*45*) and will be conducted under the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

NIH will also review on a case-by-case basis (*45*) all experiments involving the use of virus vectors in animals and will prescribe the physical and biological containment conditions appropriate for such studies. (See Section IV–E–1–b–(3)–(c).)

III–C–1–f. *Nonviral Vectors.* Organelle, plasmid, and chromosomal DNAs may be used as vectors. DNA recombinants formed between such vectors and host DNA, when propagated only in that host (or a closely related strain of the same species), are exempt from these Guidelines (see Section I–E). DNA recombinants formed between such vectors and nonviral DNA from cells other than the host species require only P1 physical containment for cells in culture since vertebrate cells in tissue culture inherently exhibit a very high level of containment. Recombinants involving viral DNA or experiments which require the use of the whole animals will be evaluated by NIH on a case-by-case basis (*45*).

III–C–2. *Invertebrate Host-Vector Systems.*

III–C–2–a. *Insect Viral Vectors.* As soon as information becomes available on the host range restrictions and on the infectivity, persistence, 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 (*45*) and will be conducted under the recommended physical containment conditions. (See Section IV–E–1–b–(3)–(c).)

III–C–2–b. *Nonviral Vectors.* Organelle, plasmid, and chromosomal DNAs may be used as vectors. DNA recombinants formed between such vectors and host DNA, when propagated only in that 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 cells from other than the host species require P1 physical containment for invertebrate cells in culture since invertebrate cells in culture inherently exhibit a very high level of containment. Experiments which require the use of whole animals will be evaluated by NIH on a case-by-case basis (*45*).

III–C–3. *Plant Viral Host-Vector Systems.* 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 (*2A*) which have relaxed circular double-stranded DNA genomes with a molecule weight of 4.5×10^6, and Bean Golden Mosaic Virus (BGMV) and related viruses with small ($<10^6$ daltons) single-stranded DNA genomes. CaMV is spread in nature by aphids, in which it survives for a few hours. Spontaneous mutants of CaMV which lack a factor essential for aphid transmission arise frequently. BGMV is spread in nature by whiteflies, and certain other single-stranded DNA plant viruses are transmitted by leafhoppers.

The DNA plant viruses have narrow host ranges and are relatively difficult to transmit mechanically to plants. For this reason, they are most unlikely to be accidentally transmitted from spillage of purified virus preparations.

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						
	Prokaryotic		Eukaryotic			Eukaryotic viral	
	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 and 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

When these viruses are used as vectors in intact plants, or propagative plant parts, the plants shall be grown under P1 conditions—that is, in either a limited access greenhouse or plant growth cabinet which is insect-restrictive, preferably with positive air pressure, (*2A*) and in which an insect fumigation regime is maintained. Soil, plant pots, and unwanted infected materials shall be removed from the greenhouse or cabinet in sealed insect-

proof containers and sterilized. It is not necessary to sterilize run-off water from the infected plants, as this is not a plausible route for secondary infection. When the viruses are used as vectors in tissue cultures or in small plants in axenic cultures, no special containment is necessary. Infected plant materials which have to be removed from the greenhouse or cabinet for further research shall be maintained under insect-restrictive conditions. These measures provide an entirely adequate degree of containment. They are similar to those required in many countries for licensed handling of "exotic" plant viruses.

The CaMV strain used as a cloning vector shall 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 provides adequate safety. Since no risk to the environment from the use of the DNA plant virus/protoplast system is envisaged, no special containment is necessary, except as described in the following paragraph.

Experiments involving the use of plant virus genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by NIH on a case-by-case basis (*45*) and will be conducted under the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

III–C–4. *Plant Host-Vector Systems Other than Viruses.* Organelle, plasmid, and chromosomal DNAs may be used as vectors. DNA recombinants formed between such vectors and host DNA, when propagated only in that host (or a closely related strain of the same species), are exempt from these Guidelines (See Section I—E). DNA recombinants formed between such vectors and DNA from cells other than the host species require P2 physical containment. The development of host-vector systems that exhibit a high level of biological containment, such as those using protoplasts or undifferentiated cells in culture, permit (2A) a decrease in the physical containment to P1.

Intact plants or propagative plant parts which cannot be grown in a standard P2 laboratory because of their large size may be grown under the P1 conditions described above in Section III–C–3, except that (i) sterilization of run-off water is required where this is a plausible route for secondary infection and (ii) the standard P2 practices are adopted for microbiological work.

III–C–5. *Fungal or Similar Lower Eukaryotic Host-Vector Systems.*

Certain certified HV1 and HV2 host-vector systems appear in Appendix D. The containment levels for these systems are given in the subsection of Section III–A. Other systems in the future may be certified as HV1 and HV2. At the time of certification, they may be added to Appendix D (and thus the containment levels for their use will be those of the subsections of Section III–A). Alternatively, at the time of their certification, another classification of containment levels for experiments using them may be assigned by NIH.

In addition to the experiments described above, the following experiments may be carried out without the eukaryotic host (Host C) having been approved as an HV1 host: DNA from Host C may be inserted into a vector and propagated in *E. coli* K–12 under P1 conditions. Subsequently, this recombinant DNA may be returned to Host C and propagated there under P1 conditions. (*43*)

Containment levels for other classes of experiments involving non-HV1 systems may be expressly approved by the Director, NIH. (See Sections IV–E–1–b–(1)–(b), IV–E–1–b–(2)–(c), and IV–E–1–b–(3)–(b).)

III–C–6. *Return of DNA Segments to a Higher Eukaryotic Host of Origin.* DNA from a higher eukaryote (Host D) may be inserted into a vector and propagated in *E. coli* K–12 under P1 containment conditions. Subsequently, this recombinant DNA may be returned to Host D and propagated under conditions of physical containment comparable to P1 and appropriate to the organism under study. (*2A*)

III–C–7. *Transfer of cloned DNA Segments to Eukaryotic Organisms*

III–C–7–a. *Transfer to Non-human Vertebrates.* DNA from any nonprohibited source [Section I–D], except for greater than one quarter of a eukaryotic viral genome, which has been cloned and propagated in *E. coli* under P1 conditions, may be transferred with the *E. coli* vector used for cloning to any eukaryotic cells in culture or to any non-human vertebrate organism and propagated under conditions of physical containment comparable to P1 and appropriate to the organism under study (*2A*). Transfers to any other host will be considered by the RAC on a case-by-case basis (*45*).

III–C–7–b. *Transfer to Higher Plants.* DNA from any nonprohibited source [Section I–D] which has been cloned and propagated in *E. coli* under P1 conditions, may be transferred with the *E. coli* vector used for cloning to any higher plant organisms (angiosperms and Gymnosperms) and propagated under conditions of physical containment comparable to P1 and appropriate to the organism under study (*2A*). Intact plants or propagative plant parts may be grown under P1 conditions described under Section III–C–3. Containment must be modified to ensure that the spread of pollen, seed or other propagules is prevented. This can be accomplished by conversion to negative pressure in the growth cabinet or greenhouse or by physical entrapment by "bagging" of reproductive structures. Transfers to any other plant organisms will be considered on a case-by-case basis (*45*).

III–D. *Complementary DNAs.* Specific containment levels are given in Section III–A–2–a (see also last column of Table III) for complementary DNA (cDNA) of viral mRNA. For the other Sections of the Guidelines, where applicable, cDNAs synthesized *in vitro* are included within each of the above classifications. For example, cDNAs formed from cellular RNAs that are not purified and characterized are included under III–A–1, shotgun experiments; cDNAs formed from purified and characterized RNAs are included under III–A–3; etc.

Due to the possibility of nucleic acid contamination of enzyme preparations used in the preparation of cDNAs, the investigator must employ purified enzyme preparations that are free of viral nucleic acid.

III–E. *Synthetic DNAs.* If the synthetic DNA segment is likely to (*2A*) yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent), the containment conditions must be as stringent as would be used for propagating the natural DNA counterpart.

If the synthetic DNA sequence codes for a harmless product, (*2A*) it may be propagated at the same containment level as its purified natural DNA counterpart. For example, a synthetic DNA segment which corresponds to a nonharmful gene of birds, to be propagated in *Saccharomyces cerevisiae*, would require P2 phyical containment plus an HV1 host-vector, or P1+HV2.

If the synthetic DNA segment is not expressed *in vivo* as a polynucleotide or polypeptide product, the organisms containing the recombinant DNA

molecule are exempt (4) from the Guidelines.

IV. Roles and Responsibilities

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

The Guidelines are intended to help the Institution, the Institutional Biosafety Committee (IBC), the Biological Safety Officer, and the Principal Investigator determine the safeguards that should be implemented. These Guidelines will never be complete or final, since all conceivable experiments involving recombinant DNA cannot be foreseen. Therefore, it is the responsibility of the Institution and those associated with it to adhere to the *purpose* of the Guidelines as well as to their specifics.

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

The following roles and responsibilities constitute an administrative framework in which safety is an essential and integral part of research involving recombinant DNA molecules. Further clarifications and interpretations of roles and responsibilities will be issued by NIH as necessary.

IV–B. *General Applicability.* The Guidelines are applicable to all recombinant DNA research within the United States or its territories which is conducted at or sponsored by an Institution that receives any support for recombinant DNA research from NIH. This includes research performed by NIH directly.

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

The Guidelines are also applicable to projects done abroad if they are supported by NIH funds. If the host country, however, has established rules for the conduct of recombinant DNA projects, then a certificate of compliance with those rules may be submitted to NIH in lieu of compliance with the NIH Guidelines. NIH reserves the right to withhold funding if the safety practices to be employed abroad are not reasonably consistent with the NIH Guidelines.

IV–C. *General Definitions.* The following terms, which are used throughout the Guidelines, are defined as follows:

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

IV–C–2. "Recombinant DNA" or "recombinant DNA molecules" means either (i) molecules which are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) DNA molecules which result from the replication of a molecule described in (i) above.

IV–C–3. "Memorandum of Understanding and Agreement" or "MUA" is a document that (i) provides to NIH or other Federal funding agency an Institution's certification that the recombinant DNA research project complies with the NIH Guidelines and (ii) contains other essential data as required in the Administrative Practices Supplement.

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

IV–C–5. "Institutional Biosafety Committee" or "IBC" means a committee that (i) meets the requirements for membership specified in Section IV–D–2, and (ii) reviews, approves, and oversees projects in accordance with the responsibilities defined in Sections IV–D–2 and –3.

IV–C–6. "NIH Office of Recombinant DNA Activities" or "ORDA" means the office within NIH with responsibility for (i) reviewing and coordinating all activities of NIH related to the Guidelines, and (ii) performing other duties as defined in Section IV–E–3.

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

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

IV–C–9. "Federal Interagency Advisory Committee on Recombinant DNA Research" means the committee established in October 1976 to advise the Secretary, HEW, the Assistant Secretary for Health, and the Director, NIH on the coordination of those aspects of all Federal programs and activities which relate to recombinant DNA research.

IV–C–10. "Administrative Practices Supplement" or "APS" means a publication to accompany the NIH Guidelines specifying administrative procedures for use at NIH and at Institutions.

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

IV–D. *Responsibilities of the Institution*

IV–D–1. Each Institution conducting or sponsoring recombinant DNA research covered by these Guidelines is responsible for ensuring that the research is carried out in full conformity with the provisions of the Guidelines. In order to fulfill this responsibility, the Institution shall:

IV–D–1–a. Establish and implement policies that provide for the safe conduct of recombinant DNA research and that ensure compliance with the Guidelines. The Institution, as part of its general responsibilities for implementing the Guidelines, may establish additional procedures, as deemed necessary, to govern the Institution and its components in the discharge of its responsibilities under the Guidelines. This may include (i) statements formulated by the Institution for general implementation of the Guidelines and (ii) whatever additional precautionary steps the Institution may deem appropriate.

IV–D–1–b. Establish an Institutional Biosafety Committee (IBC) that meets the requirements set forth in Section IV–D–2 and carries out the functions detailed in Section IV–D–3.

IV–D–1–c. Submit, for each recombinant DNA project that meets with its approval, a Memorandum of Understanding and Agreement (MUA) to the funding agency for approval and registration.

Note.—No MUA is required for experiments described in Section III–0).) All projects, however, can proceed upon IBC approval (before submission of the MUA to the funding agency) except for the following, which require prior approval by NIH (or other funding agency designated by NIH for this purpose):

IV–D–1–c–(1). Projects for which containment levels are not specified by the Guidelines or NIH,

IV–D–1–c–(2). Projects requiring P4 containment,

IV–D–1–c–(3). Reductions of containment levels for characterized or purified DNA preparations or clones (see Section III–A–3),

IV–D–1–c–(4). The first project conducted in a facility at P3 containment, or

IV–D–1–c–(5). The first project conducted by an Institution.

Note.—The MUA shall be submitted to the funding agency within 30 days of the IBC approval. If the funding agency does not routinely register recombinant DNA projects with NIH, the MUA must be submitted to NIH as well as to the funding agency. Authority to submit MUAs (or addenda) for which prior approval is not required may be delegated to the IBC chairperson. All MUAs that require NIH approval before the work can proceed shall be submitted to the NIH by the institutional official to whom the IBC is responsible.

IV–D–1–d. Take appropriate action to bring protocols into compliance when advised by NIH or other funding agency that IBC-approved projects do not conform to standards set forth in the Guidelines. This responsibility may be delegated to the IBC. (See Administrative Practices Supplement for further details).

IV–D–1–e. If the Institution is engaged in recombinant DNA research at the P3 or P4 containment level, appoint a Biological Safety Officer (BSO), who shall be a member of the IBC and carry out the duties specified in Section IV–D–4.

IV–D–1–f. Require that investigators responsible for research covered by these Guidelines comply with the provisions of Section IV–D–5, and assist investigators to do so.

IV–D–1–g. Ensure appropriate training for the IBC chairperson and members, the BSO, Principal Investigators (PIs), and laboratory staff regarding the Guidelines, their implementation, and laboratory safety. Responsibility for training IBC members may be carried out through the IBC chairperson. Responsibility for training laboratory staff may be carried out through the PI. The Institution is responsible for seeing that the PI has sufficient training, but may delegate this responsibility to the IBC.

IV–D–1–h. Determine the necessity, in connection with each project, for health surveillance of recombinant DNA research personnel, and conduct, if found appropriate, a health surveillance program for the project. [The Laboratory Safety Monograph (LSM) discusses various possible components of such a program—for example, records of agents handled, active investigation of relevant illnesses, and the maintenance of serial serum samples for monitoring serologic changes that may result from the employees' work experience. Certain medical conditions may place a laboratory worker at increased risk in any endeavor where infectious agents are handled. Examples given in the LSM include gastrointestinal disorders and treatment with steroids, immunosuppressive drugs, or antibiotics. Workers with such disorders or treatment should be evaluated to determine whether they should be engaged in research with potentially hazardous organisms during their treatment or illness.]

IV–D–1–i. Report within 30 days to ORDA any significant problems with and violations of the Guidelines and significant research-related accidents and illnesses, unless the institution determines that the PI or IBC has done so.

IV–D–2. *Membership and Procedures of the IBC.* The Institution shall establish an Institutional Biosafety Committee (IBC) meeting the following requirements:

IV–D–2–a. The IBC shall comprise no fewer than five members so selected that they collectively have experience and expertise in recombinant DNA technology and the capability to assess the safety of recombinant DNA research experiments and any potential risk to public health or the environment. At least two members (but not less than 20 percent of the membership of the committee) shall not be affiliated with the Institution (apart from their membership on the IBC) and shall represent the interest of the surrounding community with respect to health and protection of the environment. Members meet this requirement if, for example, they are officials of State or local public health or environmental protection agencies, members of other local governmental bodies, or persons active in medical, occupational health, or environmental concerns in the community. The Biological Safety Officer (BSO), mandatory when research is being conducted at the P3 and P4 levels, shall be a member (see Section IV–D–4).

IV–D–2–b. In order to ensure the professional competence necessary to review recombinant DNA activities, it is recommended that (i) the IBC include persons from disciplines relevant to recombinant DNA technology, biological safety, and engineering; (ii) the IBC include, or have available as consultants, persons knowledgeable in institutional commitments and policies, applicable law, standards of professional conduct and practice, community attitudes, and the environment; and (iii) at least one member be a nondoctoral person from a laboratory technical staff.

IV–D–2–c. The Institution shall identify the committee members by name in a report to the NIH Office of Recombinant DNA Activities (ORDA) and shall include relevant background information on each member in such form and at such times as ORDA may require. (See the Administrative Practices Supplement for further guidance.)

IV–D–2–d. No member of an IBC may be involved (except to provide information requested by the IBC) in the review or approval of a project in which he or she has been, or expects to be, engaged or has a direct financial interest.

IV–D–2–e. The Institution may establish procedures that the IBC will follow in its initial and continuing review of applications, proposals, and activities. (IBC review procedures are specified in Section IV–D–3–a.)

IV–D–2–f. Central to implementation of the Guidelines is the review of proposed experiments by the IBC. The Institutions shall submit, within 30 days of IBC approval, an MUA to NIH (ORDA), or shall otherwise register proposed experiments as specified under Section IV–D–1–c, IV–D–1–d, and IV–F. In carrying out this responsibility, the Institution shall comply with instructions and procedures specified in the Administrative Practices Supplement.

IV–D–2–g. Institutions are encouraged to open IBC meetings to the public whenever possible, consistent with protection of privacy and proprietary interests.

IV–D–2–h. Upon request, the Institution shall make available to the public all minutes of IBC meetings and any documents submitted to or received from funding agencies which the latter are required to make available to the public (e.g., NUAs, reports of Guideline violations and significant research-related accidents, and agency directives to modify projects). If comments are made by members of the public on IBC actions, the Institution shall forward to NIH both the comments and the IBC's response.

IV–D–3. *Functions of the IBC.* On behalf of the Institution, the IBC is responsible for:

IV–D–3–a. Reviewing for compliance with the NIH Guidelines all recombinant DNA research to be conducted at or sponsored by the Institution, and approving those research projects that it finds are in conformity with the Guidelines. (See Administrative Practices Supplement, II–D, for prior NIH approval requirements.) This review shall include:

IV–D–3–a–(1). An independent assessment of the containment levels

required by these Guidelines for the proposed research, and

IV–D–3–a–(2). An assessment of the facilities, procedures, and practices, and of the training and expertise of recombinant DNA personnel.

Note.—See Laboratory Safety Monograph (pages 187–190) for suggested guidance in conducting this review.

IV–D–3–b. Authorizing the Principal Investigator (PI) to proceed with the project upon receipt of proper agency approval; or authorizing the PI to proceed without agency approval to initiate or change a project for which none of the exceptions under IV–D–1–c apply.

Note.—Some examples of work that might ordinarily proceed without prior funding-agency approval are the initiation of a project at the P1 or P2 level (other than the first project at the institution). Other examples are significant changes in hosts or vectors, in the donor species or the nature of the DNA segment selected, or in the physical location of the experiments. It should be clear, however, that the funding agency must be notified of IBC approvals even when prior agency approval is not required. See the Administrative Practices Supplement for further discussion.

IV–D–3–c. Reviewing periodically recombinant DNA research being conducted at the Institution, to ensure that the requirements of the Guidelines are being fulfilled.

IV–D–3–d. Adopting emergency plans covering accidental spills and personnel contamination resulting from such research.

Note.—Basic elements in developing specific procedures for dealing with major spills of potentially hazardous materials in the laboratory are detailed in the Laboratory Safety Monograph. Included are information and references on decontamination and emergency plans. NIH and the Center for Disease Control are available to provide consultation, and direct assistance if necessary, as posted in the LSM. The Institution shall cooperate with the State and local public health departments, reporting any significant research-related illness or accident that appears to be a hazard to the public health.

IV–D–3–e. Reporting within 30 days to the appropriate institutional official and to the NIH Office of Recombinant DNA Activities (ORDA) any significant problems with or violations of the Guidelines, and any significant research-related accidents or illnesses, unless the IBC determines that the PI has done so.

IV–D–3–f. Performing such other functions as may be delegated to the IBC under Section IV–D–1.

IV–D–4. *Biological Safety Officer.* The Institution shall appoint a BSO if it engages in recombinant DNA research at the P3 or P4 containment level. The officer shall be a member of the Institutional Biosafety Committee (IBC), and his or her duties shall include (but need not be limited to):

IV–D–4–a. Ensuring through periodic inspections that laboratory standards are rigorously followed;

IV–D–4–b. Reporting to the IBC and the Institution all significant problems with and violations of the Guidelines and all significant research-related accidents and illnesses of which the BSO becomes aware, unless the BSO determines that the Principal Investigator (PI) has done so.

IV–D–4–c. Developing emergency plans for dealing with accidental spills and personnel contamination, and investigating recombinant DNA research laboratory accidents;

IV–D–4–d. Providing advice on laboratory security;

IV–D–4–e. Providing technical advice to the PI and the IBC on research safety procedures.

Note.—See Laboratory Safety Monograph for additional information on the duties of the BSO.

IV–D–5. *Principal Investigator.* On behalf of the Institution, the PI is responsible for complying fully with the Guidelines in conducting any recombinant DNA research.

IV–D–5–a. *PI—General.* As part of this general responsibility, the PI shall:

IV–D–5–a–(1). Initiate or modify no recombinant DNA research subject to the Guidelines until that research, or the proposed modification thereof, has been approved by the Institutional Biosafety Committee (IBC) and has met all other requirements of the Guidelines and the Administrative Practices Supplement (APS), and make changes to conform if the NIH Office of Recombinant DNA Activities' (ORDA's) review so requires.

Note.—No prior approval by the IBC is required for most experiments described in Section III–0.

IV–D–5–a–(2). Report within 30 days to the IBC and NIH (ORDA) all significant problems with and violations of the Guidelines and all significant research-related accidents and illnesses;

IV–D–5–a–(3). Report to the IBC and to NIH (ORDA) new information bearing on the Guidelines;

IV–D–5–a–(4). Be adequately trained in good microbiological techniques;

IV–D–5–a–(5). Adhere to IBC-approved emergency plans for dealing with accidental spills and personnel contamination; and

IV–D–5–a–(6). Comply with shipping requirements for recombinant DNA molecules. (See Section II–C for shipping requirements, Laboratory Safety Monograph for technical recommendations, and the APS for administrative instructions and procedures. The requesting laboratory must be in compliance with the NIH Guidelines and under appropriate review by its IBC, and the sending investigator must maintain a record of all shipments of recombinant DNA materials.)

IV–D–5–b. *Submissions by the PI to NIH.* The PI shall:

IV–D–5–b–(1). Submit information to NIH (ORDA) in order to have new host-vector systems certified;

IV–D–5–b–(2). Petition NIH, with notice to the IBC, for exemptions to these Guidelines (see Sections I–E–4 and I–E–5 and, for additional information on procedures, the APS); and

IV–D–5–b–(3). Petition NIH, with concurrence of the IBC, for exceptions to the prohibitions under these Guidelines (see Section I–D and, for additional information on procedures, the APS).

IV–D–5–c. *Submissions by the PI to the IBC.* The PI shall:

IV–D–5–c–(1). Make the initial determination of the required levels of physical and biological containment in accordance with the Guidelines;

IV–D–5–c–(2). Select appropriate microbiological practices and laboratory techniques to be used in the research;

IV–D–5–c–(3). Submit the initial research protocol (and also subsequent changes—e.g., changes in the source of DNA or host-vector system, which require a new or revised Memorandum of Understanding and Agreement) to the IBC for review and approval or disapproval; and

IV–D–5–c–(4). Remain in communication with the IBC throughout the conduct of the project.

IV–D–5–d. *P1 Responsibilities After Approval But Prior to Initiating the Research.* The P1 is responsible for:

IV–D–5–d–(1). Making available to the laboratory staff copies of the approved protocols that describe the potential biohazards and the precautions to be taken;

IV–D–5–d–(2). Instructing and training staff in the practices and techniques required to ensure safety and in the procedures for dealing with accidents; and

IV–D–5–d–(3). Informing the staff of the reasons and provisions for any precautionary medical practices advised or requested, such as vaccinations or serum collection.

IV–D–5–e. *P1 Responsibilities During the Conduct of the Approved Research.* The P1 is responsible for:

IV–D–5–e–(1). Supervising the safety performance of the staff to ensure that

the required safety practices and techniques are employed;

IV–D–5–e–(2). Investigating and reporting in writing to ORDA, the Biological Safety Officer (where applicable), and the IBC any significant problems pertaining to the operation and implementation of containment practices and procedures;

IV–D–5–e–(3). Correcting work errors and conditions that may result in the release of recombinant DNA materials;

IV–D–5–e–(4). Ensuring the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., purity, and genotypic and phenotypic characteristics); and

IV–D–5–e–(5). *Publications.* PIs are urged to include, in all publications reporting on recombinant DNA research, a description of the physical and biological containment procedures employed.

IV–E. *Responsibilities of NIH.*

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

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

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

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

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

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

IV–E–1–a–(4). Maintaining the Federal Interagency Advisory Committee on Recombinant DNA Research established by the Secretary, HEW, for advice on the coordination of all Federal programs and activities relating to recombinant DNA, including activities of the RAC.

IV–E–1–b. *Specific Responsibilities of the Director, NIH.* In carrying out the responsibilities set forth in this Section, the Director shall weigh each proposed action, through appropriate analysis and consultation, to determine that it complies with the Guidelines and presents no significant risk to health or the environment.

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

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

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

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

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

IV–E–1–b–(1)–(e). Permitting exceptions to the prohibited experiments in the Guidelines, in order, for example, to allow risk-assessment studies; and

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

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

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

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

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

IV–E–1–b–(2)–(d). Designating certain class 2 agents as class 1 for the purpose of these Guidelines (see Footnote 1 and Appendix B).

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

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

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

IV–E–1–b–(3)–(c). Determining appropriate containment conditions upon case-by-case analysis of experiments explicitly considered in the Guidelines but for which no containment levels have been set (see Footnote 45 in Part V; Sections III–C–1–a through –e; and Sections III–C–2 and –3);

IV–E–1–b–(3)–(d). Authorizing, under procedures specified by the RAC, large-scale experiments (i.e., involving more than 10 liters of culture) for recombinant DNAs that are rigorously characterized and free of harmful sequences (see Footnote 3 and Section I–D–6);

IV–E–1–b–(3)–(e). Lowering containment levels for characterized clones or purified DNA (see Sections III–A–3–a and –b, and Footnotes 3 and 41);

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

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

IV–E–1–b–(4). The Director shall conduct, support, and assist training programs in laboratory safety for Institutional Biosafety Committee members, Biological Safety Officers, Principal Investigators, and laboratory staff.

IV–E–1–b–(5). The Director, at the end of 36 months from the time these Guidelines are promulgated, will report on the Guidelines, their administration, and the potential risks and benefits of this research. In doing so, the Director will consult with the RAC and the Federal Interagency Committee. Public comment will be solicited on the draft report and taken into account in transmitting the final report to the Assistant Secretary for Health and the Secretary, HEW.

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

The members of the committee shall be chosen to provide, collectively, expertise in scientific fields relevant to recombinant DNA technology and biological safety—e.g., microbiology, molecular biology, virology, genetics, epidemiology, infectious diseases, the biology of enteric organisms, botany, plant pathology, ecology, and tissue culture. At least 20 percent of the members shall be persons knowledgeable in applicable law, standards of professional conduct and practice, public attitudes, the environment, public health, occupational health, or related fields. Representatives from Federal agencies shall serve as nonvoting members. Nominations for the RAC may be submitted to the NIH Office of Recombinant DNA Activities.

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

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

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

IV–E–3–a. Review and approval of Institutional Biosafety Committee (IBC) membership;

IV–E–3–b. Registration of recombinant DNA projects; and

IV–E–3–c. Review of Memoranda of Understanding and Agreement (MUAs), and approval of those that conform to the Guidelines. In so doing, ORDA shall:

IV–E–3–c–(1). Conduct an independent evaluation of the containment levels required for the research covered by these Guidelines;

IV–E–3–c–(2). Determine whether the physical and biological containment levels approved by the IBC are in accordance with the requirement of the Guidelines;

IV–E–3–c–(3). Notify Institutions and the IBC chairperson in a timely fashion when MUAs (including changes in ongoing projects) do not conform to the Guidelines, and inform them of corrective measures to be taken;

IV–E–3–c–(4). Publish in the **Federal Register:**

IV–E–3–c–(4)–(a). Announcements of Recombinant DNA Advisory Committee (RAC) meetings and agendas 30 days in advance, with publication of the Director's proposed decision for 30 days of public and Federal agency comment followed by a published response, on any action listed in Section IV–E–1–b–(1); and

IV–E–3–c–(4)–(b). Announcements of RAC meetings and agendas 30 days in advance on any action listed in Section IV–E–1–b–(2).

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

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

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

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

IV–E–4–a. Awarding no grant or contract involving recombinant DNA techniques unless a properly executed MUA has been received;

IV–E–4–b. Certifying P4 facilities, inspecting them periodically, and inspecting other recombinant DNA facilities as deemed necessary; and

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

(See Administrative Practices Supplement for additional information on the administrative procedures of ORDA and other NIH components.)

IV–F. *Registration*

IV–F–1. *Required Registration.* Institutions receiving NIH funds for recombinant DNA projects shall inform NIH of all recombinant DNA projects at the Institution. A non-NIH project, after approval by the Institutional Biosafety Committee, shall be registered with NIH within 30 days of initiation. Applications for NIH projects must be accompanied by a Memorandum of Understanding and Agreement (MUA).

For information on MUAs or equivalent documents that must be submitted for registration of recombinant DNA projects, see the Administrative Practices Supplement (APS).

IV–F–2. *Federal Agency Registration.* Institutions at which recombinant DNA research projects funded by other Federal agencies are conducted need not register such projects with NIH when the Federal agency maintains a registry and provides such information to NIH. Registration of non-NIH-funded research with the NIH Office of Recombinant DNA Activities (ORDA) is described in the APS. (The information required is similar to that in an MUA for NIH-supported research.)

IV–F–3. *Voluntary Registration and Certification.* Any institution that 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–F–4. *Disclosure of Information.* Institutions are reminded that they should consider applying for a patent before submitting information to DHEW which they regard as potentially proprietary. (Provisions for protection of proprietary information as permitted under current DHEW authorities are discussed in Part VI of these Guidelines.)

IV–G. *Compliance.* As a condition for NIH funding of recombinant DNA research, Institutions must ensure that such research conducted at or sponsored by the Institution, irrespective of the source of funding, shall comply with these Guidelines. The policies on noncompliance are as follows:

IV–G–1. All NIH-funded projects involving recombinant DNA techniques must comply with the NIH Guidelines. Noncompliance may result in (i) suspension, limitation, or termination of financial assistance for such projects and of NIH funds for other recombinant

DNA research at the Institution, or (ii) a requirement for prior NIH approval of any or all recombinant DNA projects at the Institution.

IV–G–2. All non-NIH-funded projects involving recombinant DNA techniques conducted at or sponsored by an Institution that receives NIH funds for projects involving such techniques must comply with the NIH Guidelines. Noncompliance may result in (i) suspension, limitation, or termination of NIH funds for recombinant DNA research at the Institution, or (ii) a requirement for prior NIH approval of any or all recombinant DNA projects at the Institution.

IV–G–3. Information concerning noncompliance with the Guidelines may be brought forward by any person. It should be delivered to both NIH (ORDA) and the relevant Institution. The Institution shall forward a complete report of the incident to ORDA, recommending any further action indicated.

IV–G–4. In cases where NIH proposes to suspend, limit, or terminate financial assistance because of noncompliance with the Guidelines, applicable DHEW and Public Health Service procedures shall govern.

IV–G–5. *Voluntary Compliance.* Any individual, corporation, or institution that is not otherwise covered by the Guidelines is encouraged to conduct recombinant DNA research activities in accordance with the Guidelines, through the procedures set forth in Part VI.

V. Footnotes and References

1. The reference to organisms as Class 1, 2, 3, 4, or 5 refers to the classification in the publication *Classification of Etiologic Agents on the Basis of Hazard,* 4th Edition, July 1974; U.S. Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, Office of Biosafety, Atlanta, Georgia 30333. The list of organisms in each class, as given in this publication, is reprinted in Appendix B to these Guidelines.

The Director, NIH, with advice of the Recombinant DNA Advisory Committee, may designate certain of the agents which are listed as Class 2 in the *Classification of Etiologic Agents on the Basis of Hazard,* 4th Edition, July 1974, as Class 1 agents for the Purposes of these Guidelines (see Section IV–E–1–b–(2)–(d)). An updated list of such agents may be obtained from the Office of Recombinant DNA Activities (ORDA), National Institutes of Health, Bethesda, Maryland 20205.

The entire *Classification of Etiologic Agents on the Basis of Hazard* is in the process of revision.

2. One exception to the prohibition of formation of recombinant DNAs derived from Class 3, 4, or 5 agents is that the formation of recombinant DNAs derived from Vesicular Stomatitis Virus (VSV) is not prohibited. The reason for this is explained in the "Decision Document" accompanying the proposed revised guidelines published in the **Federal Register** on July 28, 1978. However, as noted in Appendix B, a permit from the U.S. Department of Agriculture is required for the import of interstate transport of VSV. This can be obtained from USDA–APHIS, Veterinary Service, Federal Building, Hyattsville, Maryland 20782.

2A. In parts I and III of the Guidelines, there are a number of places where judgments are to be made. These include: "cells known to be infected with such agents" (Section I–D–1); "toxins potent for vertebrates" (Section I–D–2); "beyond that which occurs by natural genetic exchange" (Section I–D–3); "known to acquire it naturally" (Section I–D–5); "known to produce a potent polypeptide toxin . . . or known to carry such pathogens . . . not likely to be a product of closely linked eukaryote genes . . . shown not to contain such agents" (Section III–A–1–a–(5)–(a)); "shown to be free of disease causing microorganisms" (Section III–A–1–a–(5)–(b)); "close relatives" (Section III–C–3); and "produce a potent polypeptide toxin" (Footnote 34).

In all these cases the principal investigator is to make the initial judgment on these matters as part of his responsibility to "make the initial determination of the required levels of physical and biological containment in accordance with the Guidelines" (Section IV–D–7–a). In all these cases, this judgment is to be reviewed and approved by the Institutional Biosafety Committee as part of its responsibility to make "an independent assessment of the containment levels required by these Guidelines for the proposed research" (Section IV–D–3–a–(1)). If the IBC wishes, any specific cases may be referred to the NIH Office of Recombinant DNA Activities as part of ORDA's functions to "provide advice to all within and outside NIH" (Section IV–E–3), and ORDA may request advice from the Recombinant DNA Advisory Committee as part of the RAC's responsibility for "interpreting and determining containment levels upon request by ORDA" (Section IV–E–1–b–(2)–(a)).

3. The following types of data should be considered in determining whether DNA recombinants are "characterized" and the absence of harmful sequences has been established: (a) the absence of potentially harmful genes (e.g., sequences contained in indigenous tumor viruses or sequences that code for toxins, invasins, virulence factors, etc., that might potentiate the pathogenicity or communicability of the vector and/or the host or be detrimental to humans, animals or plants); (b) the type(s) of genetic information on the cloned segment and the nature of transcriptional and translation gene products specified; (c) the relationship between the recovered and desired segment (e.g., hybridization and restriction endonuclease fragmentation analysis where applicable); (d) the genetic stability of the cloned fragment; and (e) any alterations in the biological properties of the vector and host.

4. In section I–E, "exemptions" from the Guidelines are discussed. Such experiments are not covered by the Guidelines and need not be registered with NIH. In Section I–D on "prohibitions," the possibility of "exceptions" is discussed. An "exception" means that an experiment may be expressly released from a prohibition. At that time it will be assigned an appropriate level of physical and biological containment.

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

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

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

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

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

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

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

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

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

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

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

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

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

19A. Horsfall, F. L., Jr., and J. H. Baner (1940). *Individual Isolation of Infected*

Animals in a Single Room. J. Bact. *40,* 569–580.

20. Biological safety cabinets referred to in this section are classified as *Class I, Class II,* or *Class III* cabinets. A *Class I* is a ventilated cabinet for personnel protection having an inward flow of air away from the operator. The exhaust air from this cabinet is filtered through a high-efficiency particulate air (HEPA) filter. This cabinet is used in three operational modes: (1) with a full-width open front, (2) with an installed front closure panel (having four 8-inch diameter openings) without gloves, and (3) with an installed front closure panel equipped with arm-length rubber gloves. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater. A *Class II* cabinet is a ventilated cabinet for personnel and product protection having an open front with inward air flow for personnel protection, and HEPA filtered mass recirculated air flow for product protection. The cabinet exhaust air is filtered through a HEPA filter. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater. Design and performance specifications for *Class II* cabinets have been adopted by the National Sanitation Foundation, Ann Arbor, Michigan. A *Class III* cabinet is a closed-front ventilated cabinet of gas-tight construction which provides the highest level of personnel protection of all biohazard safety cabinets. The interior of the cabinet is protected from contaminants exterior to the cabinet. The cabinet is fitted with arm-length rubber gloves and is operated under a negative pressure of at least 0.5 inches water gauge. All supply air is filtered through HEPA filters. Exhaust air is filtered through two HEPA filters or one HEPA filter and incinerator before being discharged to the outside environment.

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

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

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

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

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

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

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

28. Thomas, M., J. R. Cameron, and R. W. Davis (1974). *Viable Molecular Hybrids of Bacteriophage Lambda and Eukaryotic DNA.* Proc. Nat. Acad. Sci. USA *71,* 4579–43583.

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

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

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

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

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

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

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

34. We are specifically concerned with the remote possibility that potent toxins could be produced by acquiring a single gene or cluster of genes. See also footnote 2A.

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

36. As classified in the 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% pure; otherwise as for shotgun experiments with eukaryotic cellular DNA.

37A. For the purpose of these Guidelines, viruses of the families *Papovaviridae, Adenoviridae,* and *Herpetoviridae* (36) should be considered as "transforming" viruses. While only certain of these viruses have been associated with cell transformation *in vivo* or *in vitro,* it seems prudent to consider all members to be potentially capable of transformation. In addition, those viruses of the family *Poxviridae* that produce proliferative responses—i.e., myxoma, rabbit and squirrel fibroma, and Yaba viruses—should be considered as "transforming."

38. >99% pure (i.e., less than 1% of the DNA consists of intact viral genomes); otherwise as for whole genomes.

39. The viruses have been classified by NCI as "moderate-risk oncogenic viruses." See "Laboratory Safety Monograph—A Supplement to the NIH Guidelines for Recombinant DNA Research" for recommendations on handling the viruses themselves.

40. HV1CV means the use of an HV1 host and a vector certified for use in an HV2 system.

41. The DNA preparation is defined as "purified" if the desired DNA represents at least 99% (w/w) of the total DNA in the preparation, provided that it was verified by more than one procedure.

42. The lowering of the containment level when this degree of purification has been obtained is based on the fact that the total number of clones that must be examined to obtain the desired clone is markedly reduced. Thus, the probability of cloning a harmful gene could, for example, be reduced by more than 10-fold when a nonrepetitive gene from mammals was being sought. Furthermore, the level of purity specified here makes it easier to establish that the desired DNA does not contain harmful genes.

43. This is not permitted, of course, if it falls under any of the Prohibitions of Section I–D. Of particular concern here is prohibition I–D–5, i.e., "Deliberate transfer of a drug resistance trait to 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 DNAs from a physically contained laboratory is carriage by an infected human. Thus the vector with an inserted DNA segment should have little or no ability to replicate or spread in humans.

For use as a vector in a vertebrate host cell system, an animal viral DNA molecule should display the following properties:

(i) It should not consist of the whole genome of any agent that is infectious for humans or that replicates to a significant extent in human cells in tissue culture. If the recombinant molecule is used to transform nonpermissive cells (i.e., cells which do not produce infectious virus particles), this is not a requirement.

(ii) It should be derived from a virus whose epidemiological behavior and host range are well understood.

(iii) In permissive cells, it should be defective when carrying an inserted DNA segment (i.e., propagation of the recombinant DNA as a virus must be dependent upon the presence of a complementing helper genome). In almost all cases this condition would be achieved automatically by the manipulations used to construct and propagate the recombinants. In addition, the amount of DNA encapsidated in the particles of most animal viruses is defined within fairly close limits. The insertion of sizable foreign DNA

sequences, therefore, generally demands a compensatory deletion of viral sequences. It may be possible to introduce very short insertions (50–100 base pairs) without rendering the viral vector defective. In such a situation, the requirement that the viral vector be defective is not necessary, except in those cases in which the inserted DNA encodes a biologically active polypeptide.

It is desired but not required that the functional anatomy of the vector be known—that is, 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, and

(iii) the template regions for the major gene product.

If possible the helper virus genome should:

(i) be integrated into the genome of a stable line of host cells (a situation that would effectively limit the growth of the vector recombinant to such cell lines) or

(ii) consist of a defective genome, or an appropriate conditional lethal mutant virus, making vector and helper dependent upon each other for propagation.

However, neither of these stipulations is a requirement.

45. Review by NIH on a case-by-case basis means that NIH must review and set appropriate containment conditions before the work may be undertaken. NIH actions in such case-by-case reviews will be published in the *Recombinant DNA Technical Bulletin.*

46. Provided the inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated on a case-by-case basis.

47. ≥99% pure; otherwise as for shotgun experiments.

VI. Voluntary Compliance

VI–A. *Basic Policy.* Individuals, corporations, and institutions not otherwise covered by the Guidelines are encouraged to do so by following the standards and procedures set forth in Parts I–IV of the Guidelines. In order to simplify discussion, reference hereafter to "institutions" are intended to encompass corporations, and individuals who have no organizational affiliation. For purposes of complying with the Guidelines, an individual intending to carry out research involving recombinant DNA is encouraged to affiliate with an institution that has an Institutional Biosafety Committee approved under the Guidelines.

Since commerical organizations have special concerns, such as protection of proprietary data, some modifications and explanations of the procedures in Parts I–IV are provided below, in order to address these concerns.

VI–B. *IBC Approval.* The NIH Office of Recombinant DNA Activities (ORDA) will review the membership of an institution's Institutional Biosafety Committee (IBC) and, were it finds the IBC meets the requirements set forth in Section IV–C–2, will give its approval to the IBC membership.

It should be emphasized that employment of an IBC member solely for purposes of membership on the IBC does not itself make the member an institutionally affiliated member of purposes of Section IV–D–2–a.

Except for the unaffiliated members, a member of an IBC for an institution not otherwise covered by the Guidelines may participate in the review and approval of a project in which the member has direct financial interest, so long as the member has not been and does not expect to be engaged in the project. Section IV–2–d is modified to that extent for purposes of these institutions.

VI–C. *Registration.* Upon approval of a recombinant DNA research project by the IBC, an institution may register the project by submitting to ORDA the information required in the Administrative Practices Supplement.

VI–D. *Certification of Host-Vector Systems.* A host-vector system may be proposed for certification by the Director, NIH, in accordance with the procedures set forth in Section II–D–2–a.

Institutions not otherwise covered by the Guidelines will not be subject to Section II–D–3 by complying with these procedures.

In order to ensure protection for proprietary data, any public notice regarding a host-vector system which is designated by the institution as proprietary under Section VI–F–1 will be issued only after consultation with the institution as to the content of the notice.

VI–E. *Requests for Exceptions, Exemptions, Approvals.* Requests for exceptions from prohibitions, exemptions, or other approvals required by the Guidelines should be requested by following the procedures set forth in the appropriate sections in Parts I–IV of the Guidelines.

In order to ensure protection for proprietary data, any public notice regarding a request for an exception, exemption, or other approval which is designated by the institution as proprietary under Section VI–F–1 will be issued only after consultation with the institution as to the content of the notice.

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

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

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

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

VI–F–4. Projects should be registered in accordance with procedures specified in the *Administrative Practices Supplement.* The following information will usually be considered publicly available information, consistent with the need to protect proprietary data:

a. The names of the institution and principal investigator.

b. The location where the experiments will be performed.

c. The host-vector system.

d. The source of the DNA.

e. The level of physical containment.

VI–F–5–a. Any institution not otherwise covered by the Guidelines, which is considering submission of data or information voluntarily to NIH, may request presubmission review of the records involved to determine whether, if the records are submitted, NIH will or

will not make part of all of the records available upon request under the Freedom of Information Act.

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

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

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

Appendix A

Section I–E–4 states that exempt from these Guidelines are "certain specified recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the Director, NIH, with advice of the Recombinant DNA Advisory Committee, after appropriate notice and opportunity for public comment (see Section IV–E–1–b–(1)–(d).) Certain classes are exempt as of publication of these Revised Guidelines. The list is in Appendix A."

Under exemption I–E–4 of these revised Guidelines are recombinant DNA molecules that are (1) composed entirely of DNA segments from one or more of the organisms within a sublist and (2) to be propagaated in any of the organisms within a sublist.

(Classification of *Bergey's Manual of Determinative Bacteriology*, eight edition. R. E. Buchanan and N. E. Gibbons, editors. Williams and Wilkins Company: Baltimore, 1974.)

Sublist A

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

Sublist B

1. *Bacillus subtilis*
2. *Bacillus licheniformis*
3. *Bacillus pumilus*
4. *Bacillus globigii*
5. *Bacillus niger*
6. *Bacillus nato*
7. *Bacillus anyloliquefaciens*
8. *Bacillus aterrimus*

Sublist C

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

Sublist D

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

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

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

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

B. Class 2 Agents:

1. Bacterial Agents

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

2. Fungal Agents

**Actinomycetes (including Nocardia* species and *Actinomyces species and Arachnia propionica)*
Blastomyces dermatitidis
Cryptococcus neoformans
Paracoccidioides 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 viruses
Influenza viruses—all types except A/PR8/34, which is in Class 1
Langat virus
Lymphogranuloma venereum agent
Measles virus
Mumps virus
Parainfluenza virus—all types except Parainfluenza virus 3, SF4 strain, which is in Class 1
Polioviruses—all types, wild and attenuated
Poxviruses—all types except *Alastrim, Smallpox, Monkey pox, and Whitepox*, which depending on experiments, are in Class 3 or Class 4
Rabies virus—all strains except *Rabies street virus*, which should be classified in Class 3 when inoculated into carnivores
Reoviruses—all types
Respiratory syncytial virus
Rhinoviruses—all types
Rubella virus
Simian viruses—all types except *Herpesvirus simiae (Monkey B virus) and Marburg virus*, which are in Class 4
Sindbis virus
Tensaw virus
Turlock virus
Vaccinia virus
Varicella virus

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

Vole rickettsia
Yellow fever virus, 17 D vaccine strain

C. Class 3 Agents:

1. Bacterial Agents

*Actinobacillus mallei**
Actinobacillus mallei*
Bartonella—all species
Brucella—all species
Francisella tularensis
Mycobacterium avium, M. bovis, M. tuberculosis
Pasteurella multocide type B ("buffalo" and other foreign virulent strains*)
*Pseudomonas pseudomallei**
Yersenia pestis

2. Fungal Agents

Coccidioides immitis
Histoplasma capsulatum
Histoplasma capsulatum var. *duboisii*

3. Parasitic Agents

Schistosoma mansoni

4. Viral, Rickettsial, and Chlamydial Agents

Alastrim, Smallpox, Monkey pox, and Whitepox, when used *in vitro*
Arboviruses—all strains except those in Class 2 and 4 (Arboviruses indigenous to the United States are in Class 3, except those listed in Class 2. *West Nile* and *Semliki Forest* viruses may be classified up or down, depending on the conditions of use and geographical location of the laboratory.)
Dengue virus, when used for transmission or animal inoculation experiments
Lymphocytic choriomeningitis virus (LCM)
Psittacosis-Ornithosis-Trachoma group of agents
Rabies street virus, when used in inoculations of carnivores (See Class 2)
Rickettsia—all species except *Vole rickettsia* when used for transmission or animal inoculation experiments
Vesicular stomatitis virus *
Yellow fever virus—wild, when used *in vitro*

D. Class 4 Agents:

1. Bacterial Agents

None.

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 encephalitas viruses*
Venesuelan equine encephalits virus, epidemic strains, when used for transmission or animal inoculation experiments
Yellow fever virus—wild, when used for transmission or animal inoculation experiments

II. Classification of Oncogenic Viruses on the Basis of Potential Hazard (2). A. Low-Risk Oncogenic Viruses:

Rous Sarcoma
SV–40
CELO
Ad7–SV40
Polyoma
Bovine papilloma
Rat mammary tumor
Avian Leukosis
Murine Leukemia
Murine Sarcoma
Mouse mammary tumor
Rat Leukemia
Hamster Leukemia
Bovine Leukemia
Dog Sarcoma
Mason-Pfizer Monkey Virus
Marek's
Guinea Pig Herpes
Lucké (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 diseas virus
Bovine infectious petechia fever
Camel pox virus
Ephermeral 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
Wesselsbron disease virus
Zyonema farciminosum (pseudofarcy)

References

1. *Classification of Etiologic Agents on the Basis of Hazard.* (4th Edition, July 1974). U.S. Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, Office of Biosafety, Atlanta, Georgia 30333.
2. *National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses* (October 1974). U.S. Department of Health, Education, and Welfare Publication No. (NIH) 75–790.
3. U.S. Department of Agriculture, Animal and Plant Health Inspection Service.

Appendix C

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

Under exemption I–E–5 of these Revised Guidelines are those recombinant DNA molecules that are propaged and maintained in cells in tissue culture and that are derived entirely from non-viral components (that is, no component is derived from a eukaryotic virus).

Appendix D

As noted above at the beginning of Section III–A, certain HV1 and HV2 host-vector systems are assigned containment levels as specified in the subsections of Section III–A. Those so classified as of publication of these Revised Guidelines are listed below.

*HV1—Unmodified laboratory strains of *Saccharomyces cerevisiae*
*HV1—The following specified strains of *Neurospora crassa* which have been modified to prevent aerial dispersion: (1) in1 (inositolless) strains 37102, 37401, 46316, 64001 and 89601.
(2) csp–1 strain UCLA37 and csp–2 strains FS 590, UCLA101 (these are conidial separation mutants).
(3) eas strain UCLA191 (an "easily wettable" mutant).
HV1—Asporogenic mutant derivatives of *B. subtilis*
These derivatives must not revert to sporeformers with a frequency greater than 10^{-8}; data confirming this requirement must be presented to NIH for certification. The following plasmids are accepted as the vector components of

* USDA permit also required for import or interstate transport.

*These follow the assigned containment levels as specified in the subsections of Section III–A with one exception. This exception is that experiments involving complete genomes of eukaryotic viruses will require P3+HV1 or P2+HV2 rather than the levels given in the subsections of Section III–A.

certified *B. subtilis* HV1 systems: pUB110, pC194, pS194, pSA2100, pE194, pT127, pUB112, pC221, pC223.

'HV2—The following sterile strains of *Saccharomyces cerevisiae*, all of which have the ste-VC9 mutation, SHY1, SHY2, SHY3, and SHY4. The following plasmids are certified for use: YIp1, YEp2, YEp4, YIp5, YEp6, YRp7, YEp20, YEp21, YEp24, YIp25, YIp26, YIp27, YIp28, YIp29, YIp30, YIp31, YIp32, and YIp33. These plasmids can be considered EK2 vectors when propagated in chi-1776.

Appendix E

As noted in the subsections of Section IV-E-1-b-(1) the Director, NIH, may take certain actions with regard to the Guidelines after public notice and RAC consideration.

Some of the actions taken to date include the following:

• The following experiment has been approved: The cloning in *B. subtilis*, under P2 conditions, of DNA derived from *Saccharomyces cerevisiae* using EK2 plasmid vectors provided that an HV1 *B. subtilis* host is used.

• Unmodified laboratory strains of *Neurospora crassa* can be used in all experiments for which HV1 *N. crassa* systems are approved provided that these are carried out at physical containment one level higher than required for HV1. However, if P3 containment is specified for HV1 *N. crassa*, this level is considered adequate for unmodified *N. crassa*. For P2 physical containment, special care must be exercised to prevent aerial dispersal of macroconidia, including the use of a biological safety cabinet.

• P2 physical containment shall be used for DNA recombinants produced between members of the genera *Streptomyces* and *Micromonospora* except for those species which are known to be pathogenic for man, animals or plants.[2A].

• Cloned desired fragments from any non-prohibited source may be tranferred into *Agrobacterium tumefaciens* containing a Ti plasmid (or derivatives thereof), using a nonconjugative *E. coli* plasmid vector coupled to a fragment of the Ti plasmid and/or the origin of replication of an *Agrobacterium* plasmid, under containment conditions one step higher than would be required for the desired DNA in HV1 systems (i.e. one step higher physical containment than that specified in the subsections of Section III-A). Transfer into plant parts or cells in culture would be permitted at the same containment level (one step higher).

• *Bacillus subtilis* strains that do not carry an asporogenic mutation can be used as hosts specifically for the cloning of DNA derived from *E. coli* K-12 and *Streptomyces coelicolor* using NIH-approved *Staphylococcus aureus* plasmids as vectors under P2 conditions.

• *Streptomyces coelicolor* can be used as a host for the cloning of DNA derived from *B. subtilis*, *E. Coli* K-12, or from *S. aureus* vectors that have been approved for use in *B. subtilis* under P2 conditions.

• Certain cloned segments of *Anabena* DNA may be transferred into *Klebsiella* under P2 physical containment.

• Permission is granted to clone foot-and-mouth disease virus in the EK1CV host-vector system consisting of *E. coli K-12 and the vector pBR322, all work to be done at the Plum Island Animal Disease Center.*

Dated: January 23, 1980.

Donald S. Fredrickson,

Director, National Institutes of Health.

[FR Doc. 80-2822 Filed 1-28-80; 8:45 am]

BILLING CODE 4110-08-M