Development of the National Institutes of Health Guidelines for Recombinant DNA Research

BERNARD TALBOT, MD, PhD

Tearsheet requests to Dr. Bernard Talbot, Deputy Director, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bldg. 31, Rm. 7A03, Bethesda, Md. 20205.

SYNOPSIS

Recombinant DNA is a technique of major importance in basic biomedical research and, increasingly, in industrial applications. Although the risks of this research remain hypothetical, scientists working in the field have spearheaded discussions of safety.

The original National Institutes of Health (NIH) Guidelines for Recombinant DNA Research were issued in June 1976. They assigned each type of recombinant DNA experiment a specific level of "physical containment" and of "biological containment." Responsibility for overseeing the application of the guidelines belongs to the NIH Recombinant DNA Advisory Committee (RAC) — composed of scientists and laymen, including non-voting representatives from many Federal agencies—and local institutional biosafety committees at each university where recombinant DNA research is conducted.

The NIH guidelines were subsequently adopted by other Federal agencies, but congressional proposals aimed at extending the guidelines to private industry did not result in national legislation. Some States and localities regulate recombinant DNA research, however, and many private companies have voluntarily submitted information on their recombinant DNA work for RAC and NIH approval.

The NIH guidelines underwent a major revision in December 1978 and have been revised approximately every 3 months since then. NIH supports experiments to assess recombinant DNA risks and publishes and updates a plan for a risk assessment program.

DEOXYRIBONUCLEIC ACID (DNA) makes up the genetic material of all cells and determines hereditary characteristics. Recombinant DNA is a technique, first reported (*1,2*) in 1972, that allows the transfer of genes from cells of one species to cells of another species in the laboratory.

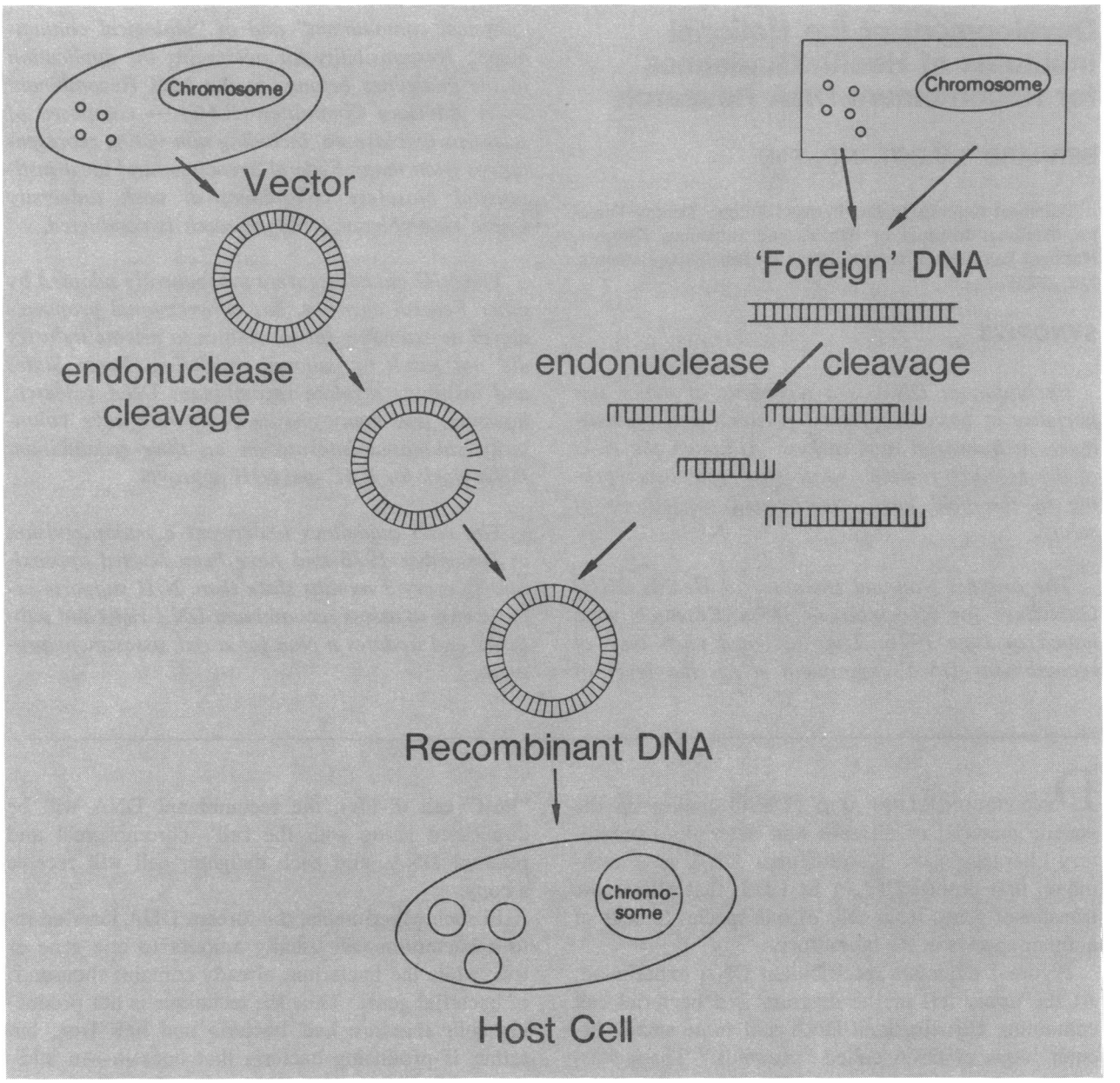
Figure 1 depicts a recombinant DNA experiment. At the upper left of the diagram is a bacterial cell containing chromosomal DNA and some small, circular loops of DNA called "plasmids." These plasmids can be isolated from the bacterial cell and cut open by an enzyme known as a restriction endonuclease. At the upper right of the diagram is another cell that can be from any species—bacteria, fly, frog, or man. The DNA of this cell can also be extracted and treated by a restriction endonuclease to yield pieces of DNA. When the material from both cells is mixed in a test tube, one of the products is a plasmid from the bacterial cell that carries a piece of DNA from the other cell. As indicated at the bottom of the diagram, this recombinant DNA can be inserted back into a bacterial cell. When the "host" cell divides, the recombinant DNA will be duplicated along with the cell's chromosomal and plasmid DNA, and each daughter cell will receive a copy.

In such experiments, the foreign DNA inserted into a bacterium will usually amount to one gene or less, while the bacterium already contains thousands of bacterial genes. Thus the technique is not producing cells that are half bacteria and half frog, but rather is producing bacteria that contain—in addition to all their normal DNA—less than one part in a thousand of additional DNA that originally derived from another species.

Uses of Recombinant DNA Technology

By introducing a particular piece of DNA into a bacterium and then culturing the bacterial cells, one can produce large amounts of the desired DNA segments for study. This technique has been, and continues to be, widely used, in thousands of laboratories throughout the world, to produce DNA that

Figure 1. A recombinant DNA experiment. A small loop of DNA from a bacterium (upper left) is cleaved by an enzyme and mixed with similarly treated DNA from another cell. The opened bacterial plasmid takes up a piece of the "foreign" DNA, forming a recombinant DNA plasmid that is then inserted into a "host" bacterium. When the host divides, each daughter cell will receive a copy of the recombinant DNA as well as the host's genetic material



scientists then analyze to determine the precise structure of specific genes. Such studies have led to a major finding about the organization of DNA in eukaryotic cells: the existence of "intervening," or "intron," sequences (*3–5*). Much new information is arising from recombinant DNA experiments that is currently of great importance in basic biomedical research, and that promises to be of still greater importance in the future diagnosis, treatment, and prevention of many diseases.

If the inserted recombinant DNA in the cell is transcribed into messenger RNA (ribonucleic acid) and then translated into protein, a whole new range of possibilities opens up. Major successes have been reported in the past few years, leading to the production by bacteria of mammalian proteins such as somatostatin (*6*), insulin (*7–9*), growth hormone (*10,11*) and interferon (*12,13*). Techniques are being perfected to increase the yields of bacterial production of such proteins. Theoretically, any protein can be made in bacteria. Recombinant DNA promises to yield huge amounts of such scarce prod-

ucts as biologically active peptides (*14*) and viral antigens for use as vaccines (*15–17*), at much lower cost than can be achieved today.

Outside the pharmaceutical industry, many other uses for micro-organisms into which recombinant DNA has been inserted are being explored. Among these uses are:

- Chemical production—inserting genes into bacteria so that they can synthesize various industrially important organic chemicals, such as ethylene oxide and ethylene glycol.
- Energy production—inserting genes into bacteria to enable them to convert plants or sewage into methane, methanol, ethanol, hydrogen, or other compounds that could be burned as fuels.
- Metal extraction—inserting genes into bacteria to aid in the extraction of desired metals from ores.

There has been intense press interest in the industrial uses of micro-organisms into which recombinant DNA has been inserted (*18–24*).

Beyond the insertion of recombinant DNA into micro-organisms, a whole other class of uses, just beginning to be explored, involves the insertion of recombinant DNA into higher organisms. There have already been numerous instances of recombinant DNA's being added to, and expressing protein products in, the cells of higher organisms in tissue culture. A future goal is the insertion of nitrogen fixation genes into agriculturally important plants, eliminating the need for fertilizers. Ultimately, it should be possible to alter the genetic constitution of higher animals and man to cure inherited disorders.

Safety Concerns

The benefits of recombinant DNA research are already many; the risks remain hypothetical. Recombinant DNA experiments have now been performed for over 10 years, and millions of recombinant DNA clones have been produced in thousands of laboratories throughout the world. To date, no actual hazard has been demonstrated. But because of concern about possible dangers of recombinant DNA molecules, scientists working in this field have from the beginning spearheaded discussions of safety.

Both the promise and the possible hazards of recombinant DNA were discussed at a 1973 Gordon Conference. Those present voted that a letter be sent to the National Academy of Sciences and be published (*25*), suggesting that the academy "consider this problem and recommend specific actions or guidelines."

In response to this initiative, the academy formed a committee of distinguished scientists, chaired by Dr. Paul Berg of Stanford University. These scientists prepared a letter (*26*) that appeared simultaneously in Science, Nature, and the Proceedings of the National Academy of Sciences.

First, the letter proposed that "until the potential hazards of such recombinant DNA molecules have been better evaluated or until adequate methods are developed for preventing their spread, scientists throughout the world join with the members of this committee in voluntarily deferring [certain] experiments." This request by scientists for a voluntary "moratorium" on such work while questions of public safety were further evaluated was widely hailed in the press.

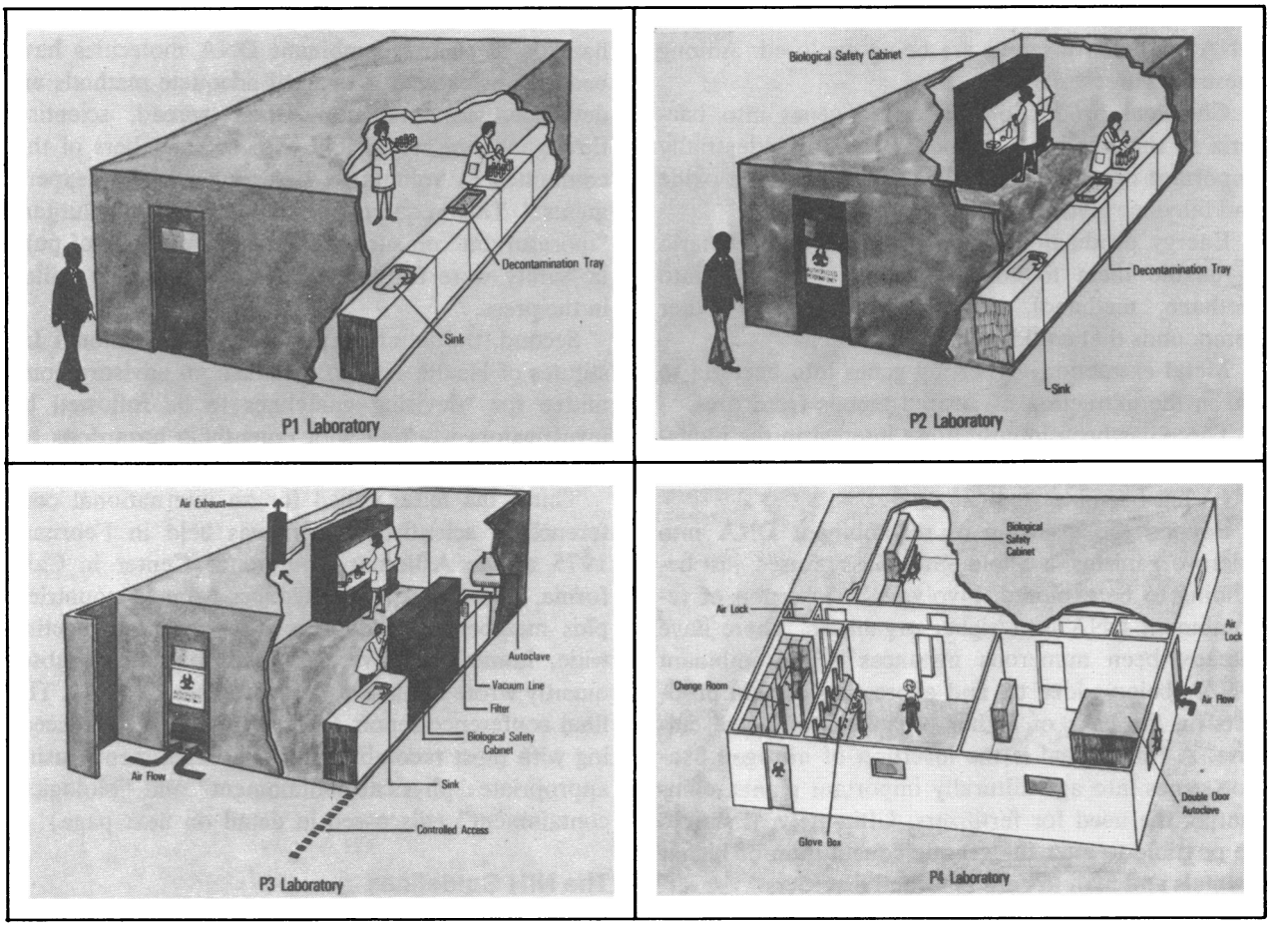
Second, the letter proposed that the National Institutes of Health (NIH) establish an advisory committee for "devising guidelines to be followed by investigators working with potentially hazardous recombinant DNA molecules."

Third, the letter called for an international conference of scientists, which was held in February 1975 at the Asilomar Conference Center in California. There were 150 attendees from 15 countries, plus members of the press who gave the meeting wide, immediate coverage. Two journalists subsequently wrote books on the conference (*27,28*). The final conference report (*29*) recommended proceeding with most recombinant DNA experiments, using appropriate "physical containment" and "biological containment" (discussed in detail on next page).

The NIH Guidelines

The first meeting of the NIH Recombinant DNA Advisory Committee (RAC)—formed in response to the letter of Berg and his associates—was held the day after the Asilomar conference. (Minutes of all RAC meetings are available from the Office of Recombinant DNA Activities, NIH, Bldg. 31, Rm. 3B10, Bethesda, Md. 20205.) After a series of meetings, the RAC in December 1975 adopted its proposed guidelines for recombinant DNA research carried out with NIH funding. When the NIH Director at the time, Dr. Donald Fredrickson, received the proposal, he called a meeting of his Director's Advisory Committee, to which he invited many distinguished scientific and public representatives. (The full transcript of this February 1976 meeting, and all letters of comment on the proposed guidelines, form the bulk of Volume 1 of what is now a seven-volume massive public record (*30–36*) of the history of the NIH guidelines. The first five volumes in this series can be purchased from the Superintendent of Documents, U.S. Government Printing Office,

Figure 2. These diagrams illustrate the four levels of physical containment specified for various types of recombinant DNA experiments by the NIH guidelines. The P1 level is that of a hospital microbiology laboratory. Each higher level adds more special practices, equipment, and installations, culminating in the P4 laboratory with its air-tight biological safety cabinets and experiments performed through glove ports



Washington, D.C. 20402, or viewed in some 600 public libraries of the GPO depository system. Volumes 6 and 7 are available from the Office of Recombinant DNA Activities at NIH.) Following an analysis of the comments and suggestions received at the February 1976 meeting and afterwards, Fredrickson addressed a number of questions to the RAC for discussion at its April 1976 meeting. After an analysis of the RAC's responses, Fredrickson decided on the final form of the NIH guidelines, promulgated in July 1976 (*37*).

The original guidelines included a list of prohibited experiments and described in great detail four sets of special practices, equipment, and laboratory installations that defined four levels of physical containment: P1, P2, P3, and P4 (fig. 2). P1 corresponds to the microbiology diagnostic laboratories, existing in all hospitals, where infectious microorganisms isolated from patients are grown and analyzed. P2 adds more practices and equipment—most important, the use of biological safety cabinets for certain operations. P3 adds still more special practices, equipment, and laboratory installations; most important, the entire laboratory is operated with an inward air flow, as though it were a giant hood. P4 laboratories have many special engineering features. All experiments are confined to air-tight biological safety cabinets, and scientists perform their work through glove ports. In addition, a whole set of secondary barriers exists.

P1 to P4 are levels of physical containment; however, a major advance resulting from the Asilomar conference was the concept of biological containment—the use, in experiments, of micro-organisms with limited ability to survive outside the very special conditions that are maintained in the laboratory. Most recombinant DNA experiments at present are being done with the harmless bacterium *Escherichia coli,* strain K-12. Its use, together with that of certain specified plasmids or bacteriophage viruses into

which the foreign DNA is inserted, constitutes what is called the EK1 level of biological containment. By further modifying *E. coli* K-12 to render the bacteria much less likely to survive, were they to escape from the laboratory (for example, by making them dependent for survival on certain nutrients that are supplied in the laboratory but that do not occur in significant concentrations in nature, and by making the modified bacteria sensitive to sunlight and to bile acids), and by requiring data on survivability to be submitted to NIH and approved by the RAC, one arrived at what were called the EK2 and EK3 levels of biological containment.

Having defined four levels of physical containment and three levels of biological containment, the guidelines then went on to specify levels of physical and biological containment required for each of many different kinds of experiments. Finally, the guidelines discussed the roles and responsibilities of the scientist, his or her university, the university's institutional biosafety committee (which in most cases already existed to oversee other potential hazards), and the NIH.

After their promulgation in 1976, the NIH guidelines were adopted by other Federal agencies. (The three major Federal agencies funding recombinant DNA research are NIH, the National Science Foundation, and the Department of Agriculture.)

In July 1976, Senators Jacob Javits and Edward Kennedy wrote to President Gerald Ford, urging that "every possible measure be explored for assuring that the NIH guidelines are adhered to in all sectors of the research community." In his reply to the two Senators, President Ford described the creation of the Federal Interagency Advisory Committee on Recombinant DNA Research. This committee has met periodically since 1976 and consists of members from all Federal agencies that either fund or might regulate recombinant DNA research. In 1977, the committee recommended new national legislation to extend the NIH guidelines to private industry.

In the first session of the 95th Congress, which lasted through 1977, 16 different bills on the topic of recombinant DNA were introduced, and extensive hearings were held. More than 100 witnesses appeared before the Senate Subcommittee on Health and Scientific Research; the Senate Subcommittee on Science, Technology, and Space; the House Subcommittee on Health and Environment; and the House Subcommittee on Science, Research, and Technology. There was great disagreement on a number of provisions of the proposed recombinant DNA bills, and none ever reached the floor of the full House or Senate. There is, therefore, no national law making the NIH guidelines mandatory for private industry.

In the absence of national legislation, a number of States and localities have acted. In Cambridge, Mass., in 1976, the city council called for a 6-month moratorium on all P3 and P4 research at Harvard University and the Massachusetts Institute of Technology while an appointed experimental review board studied the problem. The board consisted of a former Cambridge mayor and owner of a heating oil business, a community worker, a hospital nurse, an engineer, a practicing physician, a social worker, and a professor of urban policy. None of the members knew anything about recombinant DNA before they were appointed. They heard more than 75 hours of testimony and finally issued their report in January 1977, recommending that recombinant DNA research be allowed in Cambridge, basically under the NIH guidelines, with a few added restrictions. The report was adopted by the Cambridge city council in February 1977. Other local jurisdictions that have made the NIH guidelines mandatory are Princeton, N.J., Amherst, Mass., Waltham, Mass., Berkeley, Calif., and Emeryville, Calif. New York State and Maryland have also enacted such legislation.

December 1978 Guidelines Revision

In December 1978 a revision of the NIH guidelines was issued. The revision involved many steps. First, the RAC worked, at a number of meetings during the spring of 1977, to produce draft revisions. A workshop held in Falmouth, Mass., in June 1977 (*38*) led to a consensus of experts that *E. coli* K-12 is a harmless organism and cannot be converted into a pathogen by the insertion of recombinant DNA. Revised guidelines proposed by the RAC were published in the Federal Register in September 1977 (*39*) and sent out widely for public comment. At the NIH Director's Advisory Committee meeting in December 1977, many witnesses gave their views of the proposed revisions. Additional scientific meetings were held, focusing especially on the risks of recombinant DNA experiments involving viruses (*40*) and plant pathogens (*41*). Then, after much further analysis, a new set of proposed revised guidelines was published in July 1978. This document (*42*), which amounted to 136 pages in the Federal Register, had three parts: the new proposed guidelines; a "decision document" explaining in detail the proposed changes and the reasons for them, as well as why certain suggested changes were not adopted; and an environmental impact assessment. The document was mailed to more than 2,500 persons who had communicated their interest in the issue to NIH,

with a 60-day period allowed for public comment; 170 responses were received. In addition, a public hearing was held in September 1978, chaired by the General Counsel of the Department of Health, Education and Welfare.

After careful analysis of all comments received, the revised guidelines were promulgated on Dec. 22, 1978 (*43*), accompanied by a new decision document and an environmental impact assessment. Some of the major changes in the December 1978 guidelines, as compared with the original, were:

1. In general, experiments were assigned lower levels of required containment.
2. Certain classes of experiments deemed of the lowest potential hazard were exempted entirely from the guidelines.
3. Increased representation was mandated on local institutional biosafety committees (which oversee recombinant DNA research at individual institutions) and on the RAC.
4. Procedures were built into the guidelines for changing them in the future.

The RAC had originally been a 14-member committee composed entirely of scientists. At the RAC's own suggestion, two laymen were added to the committee in 1976: a professor of government and a bicethicist. At the time of the 1978 guidelines revision, the RAC was expanded to 25 voting members, with the requirement that at least 6 members "be persons knowledgeable in applicable law, standards of professional conduct and practice, public attitudes, the environment, public health, occupational health, or related fields." Also, scientists representing many different backgrounds were added as members, and all relevant Federal agencies were given nonvoting membership. Now 15 agencies are represented, including the National Science Foundation, Department of Agriculture, Environmental Protection Agency, and Occupational Safety and Health Administration.

Local institutional biosafety committees also underwent an expansion as a result of the 1978 guidelines revision. Membership on each of these committees must now include at least two persons, not affiliated with the institution, who represent the interests of the surrounding community with respect to health and protection of the environment.

Subsequent Guidelines Revisions

Perhaps the major change in the December 1978 guidelines was that a process was built into them for further change. Anyone wishing to suggest a revision of the guidelines may submit it to NIH. It is then published in the Federal Register, at least 30 days before a regular meeting of the RAC, for public comment. The suggested revision and all written comments received are considered by the RAC at an open meeting; members of the public wishing to speak on the subject are given the opportunity to do so. Following the discussion, the RAC votes on whether or not to recommend the revision. After the meeting, the responsible Federal official (before June 1981 this was the NIH Director; since then, the responsibility has been delegated to the Director of the National Institute of Allergy and Infectious Diseases) promulgates his final decision on the RAC recommendations in the Federal Register. In this fashion, the guidelines have been incrementally modified approximately every 3 months since December 1978 (*44–56*), the most recent revision appearing in the Federal Register on Aug. 27, 1982 (*56*). (Copies of this revision, and of any future ones, can be obtained from the NIH Office of Recombinant DNA Activities.)

The major differences between the current guidelines and those issued in December 1978 are:

1. Many more classes of experiments are exempted entirely from the current guidelines.
2. In general, covered experiments are assigned lower levels of required containment under the current guidelines.
3. The current guidelines lessen requirements for prior approval of many classes of experiments.
4. The current guidelines have been reorganized and simplified.

The guidelines continue to be mandatory for institutions receiving NIH funding. Certain experiments continue to require prior review by the local institutional biosafety committee, and some experiments also require prior approval by NIH.

Risk Assessment

Scientific support for the changes that have been made in the guidelines over time has come in part from risk assessment experiments supported by the NIH. In April 1979, NIH issued a proposed plan for a program to assess risks of recombinant DNA research (*57*). Following review of the proposal by the RAC and analysis of public comments received, a final plan was issued in September 1979 (*58*). A risk assessment workshop was held in April 1980 (*59*), and a proposed update of the risk assessment plan was issued in September 1980 (*60*) and made final in June 1981 (*61*). A new proposed update was issued for public comment in December 1982 (*62*).

The Guidelines and the Private Sector

The original NIH guidelines dealt only with institutions receiving Federal funds for recombinant DNA research and said nothing about the private sector. In the absence of legislation mandating compliance by industry with the guidelines, NIH provided a means for voluntary compliance. A new section—Part VI, "Voluntary Compliance"—was formally added to the NIH guidelines in January 1980, following its endorsement by the Federal Interagency Advisory Committee and the RAC.

Under Part VI, private companies may register experiments with NIH, seek clarification of the guidelines, and receive NIH certification of new host-vector systems. (Part VI also specifies how NIH will protect proprietary information voluntarily submitted to it.) In addition, private companies may submit information about the membership of their institutional biosafety committees to NIH, which will verify that the committees meet the requirements of the NIH guidelines. (To date, 51 companies have registered their committees with NIH.)

The 1978 guidelines stated that certain recombinant DNA experiments involving more than 10 liters in volume required prior approval by the NIH Director. A number of proposals to exceed 10 liters were voluntarily submitted by industry to NIH for review, were recommended for approval by the RAC after careful study, and were finally approved by NIH. These proposals included large-scale production of human insulin, growth hormone, somatostatin, and interferon. In April 1980 NIH issued physical containment recommendations for large-scale recombinant DNA work (*63*).

Summary

Recombinant DNA techniques are a major scientific advance, used widely in biomedical research and increasingly in industrial applications. Benefits of these techniques are being produced in thousands of laboratories throughout the world, while scientific data along a number of lines indicate that the potential hazards were initially overestimated. The NIH Guidelines for Recombinant DNA Research provide widely accepted safety standards, continuously evolving in response to the recommendations of scientists and laymen.

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