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DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

DRAFT

RECOMBINANT DNA ADVISORY COMMITTEE

MINUTES OF MEETING¹

APRIL 11, 1983

The Recombinant DNA Advisory Committee (RAC) was convened for its twenty-seventh meeting at 9:00 a.m. on April 11, 1983, in Building 31C, Conference Room 6, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20205. Dr. Kenneth Berns (Chairman), University of Florida, presided. In accordance with Public Law 92-463, the meeting was open to the public. The following were present for all or part of the meeting:

Committee members:

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|-----------------|------------------|--------------------------|
| Winston Brill | King Holmes | Robert Mitchell |
| Royston Clowes | Arthur Landy | Elena Nightingale |
| L. Albert Daloz | Myron Levine | Mark Saginor |
| Nina Fedoroff | Werner Maas | John Scandalios |
| David Friedman | David Martin | Pieter Wensink |
| Susan Gottesman | Gerard McGarrity | William J. Gartland, Jr. |
| Jean Harris | John McGonigle | (Executive Secretary) |
| John Harvin | Robert McKinney | |

A Committee roster is attached (Attachment).

Ad hoc consultant:

Ann Vidaver, University of Nebraska

Non-voting members:

- George Duda, Department of Energy
- Richard Green, Veterans Administration
- Morris Levin, Environmental Protection Agency
- Herman Lewis, National Science Foundation
- Henry Miller, National Center for Drugs and Biologics, FDA
- Sue Tobin, Department of Agriculture
- William Walsh, Department of State

¹The RAC is advisory to the NIH, and its recommendations should not be considered as final and accepted. NIH action on many of these recommendations was published in the Federal Register on June 1, 1983 (48 FR 24556). The Office of Recombinant DNA Activities should be consulted for NIH policy on specific issues.

Other National Institutes of Health staff:

Stanley Barban, NIAID
 W. Emmett Barkley, OD
 Annette Bower, OD
 Becky Connors, NIAID
 Herschel Cribb, OD
 Sister Jean Dechant, OD
 Henry Lewis, OD
 Charles McCarthy, OD
 Elizabeth Milewski, NIAID
 Stan Nagle, NIAID
 Bernard Talbot, NIAID
 Tossia Taylor, OD
 Robert Wiseberg, NICHD

Others:

Ed Applebaum, AgriGenetics Corporation
 Fred Betz, Environmental Protection Agency
 Irene Brandt, Eli Lilly & Company
 David Brantley, New England Nuclear
 Robert Brey, Genex Corporation
 Steve Budiansky, Nature Magazine
 Louise Cannon, Stenotech, Inc.
 Chia T. Chen, OSHA, Department of Labor
 Aileen Compton, Smith Kline & French Laboratories
 Diane Darneille, Schering-Plough Corporation
 Paula Dwyer, McGraw Hill
 Charles Eby, Monsanto Company
 Robert Eltz, Monsanto Company
 Pat Fallon, Hoffmann-LaRoche, Inc.
 John Galet, Schering-Plough Corporation
 Charles Gauth
 Richard Geoghegan, E. I. DuPont De Nemours and Company
 Jim Gideon, National Institute for Occupational Safety & Health
 Michael Goldberg, Cetus Madison Corporation
 Allen Goldhammer, Industrial Biotechnology Association
 Phil Hilts, Washington Post
 Dorothy Jessop, Department of Agriculture
 Mary Jane Johnson, Pall Corporation
 Attila Kadar, Food and Drug Administration
 Rihito Kimura, Kennedy Institute
 D. S. Mabry, Pfizer, Inc.
 Bhushan Mandava, Environmental Protection Agency
 Kenneth Martinez, National Institute for Occupational Safety & Health
 James McCullough, Library of Congress
 Bernard Mlynczak, Monsanto Company
 Bill Muth, Eli Lilly & Company
 Claire Nader

Mike Norton, British Embassy
Tom O'Brien, National Bureau of Standards
Doug Podolsky, Genetic Engineering Letter
Tabitha Powlege, Biotechnology Magazine
Harvey Price, Industrial Biotechnology Association
Steve Probyn, Harvard University
Marvin Rogul, Environmental Protection Agency
Mark Segel, Environmental Protection Agency
Jeff Trehitt, McGraw Hill
Charles Turbyville, NIH Week
Joseph Van Houten, Schering-Plough Corporation
Al Waitz, DNAX Corporation
Stephanie Zobrist, Embassy of Switzerland

I. CALL TO ORDER AND OPENING REMARKS

The Chairman, Dr. Kenneth Berns, called the meeting to order at 9:00 a.m., on April 11, 1983. He introduced the newly appointed members of the committee: Dr. Royston Clowes of the University of Texas at Dallas; Dr. John McGonigle of Santa Monica, California; and Dr. Susan Gottesman of the National Cancer Institute, National Institutes of Health. Dr. Berns said two newly appointed members could not attend the April 11 meeting. They are Dr. Wolfgang Joklik of Duke University Medical Center and Dr. Mark Mills of Vincennes, Indiana. Dr. Berns welcomed the newly appointed members and wished them success in their tenure on the RAC.

II. MINUTES OF THE OCTOBER 25, 1982, MEETING

Dr. Berns called on Dr. Harris to review the draft minutes (tab 1105) of the October 25, 1982, RAC meeting. Dr. Harris said the draft minutes are substantively correct.

Dr. Nightingale said a sentence in Section III, CDC/NIOSH Draft Report on Medical Surveillance, is incomplete. The sentence reads:

"Dr. Nightingale said she could not determine if the report merited publication and, thus, could not make a recommendation on whether the CDC/NIOSH report should be published in the Recombinant DNA Technical Bulletin."

She said the reason she could not make a recommendation concerning publication was because she did not know the editorial policy of the journal. She asked that this be clarified.

Dr. Maas said a correction should be made in Section VIII, Part A, Request to Clone a Toxin Gene from E. coli, where Shiga toxin is described as being "endotoxic." Dr. Maas said Shiga toxin is not "endotoxic," rather it is "enterotoxic" in that it causes fluid release from the jejunum. It was also noted that "Shiga" should be capitalized.

Dr. Harris moved acceptance of the minutes as amended. Dr. Landy seconded the motion. By a vote of fifteen in favor, none opposed, and one abstention, the minutes were accepted.

III. REPORT ON SOCIAL AND ETHICAL ISSUES OF GENETIC ENGINEERING WITH HUMAN BEINGS

Dr. Berns called on Mr. Mitchell to begin discussion of the "Report on the Social and Ethical Issues of Genetic Engineering with Human Beings" (tabs 1091, 1092). Mr. Mitchell said this report, entitled "Splicing Life," was

an outgrowth of a June 1980 request to President Carter by three general secretaries of the three principal U.S. religions. The religious leaders said genetic engineering raises fundamental concerns about the nature of human life and the dignity and worth of the individual. They asked that the ethical and social implications of genetic engineering of human beings be evaluated. They questioned whether government oversight was adequate. The secretaries did not expect the private sector to resolve these types of problems and thought that a broader context was required than was established in the commercial, medical, and scientific communities.

The President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research responded and began a study in 1980. The final draft of the President's Commission report was distributed at a hearing held by the Subcommittee on Investigations and Oversight of the Committee on Science and Technology of the United States House of Representatives, chaired by Representative Albert Gore (D-Tenn), on November 16, 17, and 18, 1982. Testimony was presented on the state of the art and concepts in genetic engineering. Most of the panelists agreed greater oversight was required for the ethical and social issues. Mr. Mitchell said Mr. Gore said that he intends to introduce legislation to create an independent genetic engineering commission.

Dr. Talbot said the Public Health Service (PHS) intends to publish "Splicing Life" or a summary of it in the Federal Register for public comment.

Mr. Mitchell then described the report, "Splicing Life." He said chapter one delineates general statements and concepts, describes the history of genetic engineering and describes RAC's role. The report acknowledges that no injury has occurred; rather it focuses on ethical concerns about specific applications. The report points out that a new tool and new power have been acquired.

Mr. Mitchell said chapter two describes genetic engineering techniques such as cell fusion, genetic screening, gene therapy, and gene surgery.

Chapter three discusses social and ethical issues. The chapter questions whether these issues can be resolved by a formula of balancing benefits and risks. Considerable language is devoted to the topic of "are we playing God," and if so, to what effect? Could this technology affect the concept of humanness? Could this affect concepts of self? What will be the impact of genetic engineering on family and parental rights? Would genetic alteration of an individual differentiate that person sufficiently to lessen bonds of family and kinship? Should individuals have the awesome power of manipulating the basic substance of life? What would be the impact on evolution? Would the gene pool be affected?

Mr. Mitchell said the chapter examines the ethics of creating new life forms. Would this constitute an interference with nature? The spectre of mixing human and non-human genes is raised; it is suggested that this possibility be discussed. There are also questions raised as to the appropriateness of modifying germ cells as distinguished from somatic cell line modification. The consequences of genetic screening are mentioned.

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Mr. Mitchell said chapter four concludes that currently no governmental agency has adequate oversight of ethical and social issues. The Commission supports a continuing oversight process with substantial federal government involvement and coordination among agencies, with some involvement of the private sector. The report offers a number of specific suggestions as to the constitution of an oversight group and the functions the group should perform. The group should: (1) educate the scientific community to be fully aware of the social and ethical implications of scientific activity and educate the general public in the science; (2) provide general oversight and leadership as well as direct liaison with other agencies; (3) serve as an intermediary between the biomedical and scientific community and the public; (4) operate on a scientifically sound basis; (5) treat genetic engineering in as unified a framework as possible; and (6) be separated from any sponsoring functions so that no conflicts of interest will occur.

Mr. Mitchell said "Splicing Life" commented on the RAC and its activities. He said the report recognizes RAC's contribution and success, and acknowledges that certain benefits would be gained from building on the history of the RAC. The report also alludes to comments by former NIH Director, Donald Fredrickson, concerning his suggestion that there be a third generation RAC, i.e., representing a broader community.

Mr. Mitchell then commented on the recommendations of the report, "Splicing Life." He felt oversight responsibilities should reside in one group. One single oversight group would provide an opportunity for a complete interchange of ideas. Mr. Mitchell said the report implies that persons having a humanities background may be better able to identify and resolve social and ethical issues. He felt, however, that the group should have some members with pragmatic scientific experience since genetic engineering is grounded in technology. To avoid political influences, the group should retain a degree of independence, yet have access to key decision makers.

Dr. Harris endorsed the concept that one group should provide oversight. She noted that RAC, if it were to become the oversight body for human genetic engineering, would be transformed. Issues beyond the laboratory biohazards RAC considers its primary focus would have to be considered. Dr. Harris said there are several advantages for RAC assuming this additional responsibility. She expressed reservations about severing an oversight body for human genetic engineering from the scientific community and from the NIH which not only functions as a planning and implementing component but also as an interpreter to the community at large. Moreover, RAC has a history of responsibility in considering the public good as it relates to biohazards in biotechnology and has a record of unbiased, dispassionate review.

Dr. Saginor also endorsed the concept of having a single oversight group.

Dr. Nightingale said the ethical concerns associated with genetic engineering are quite different from biosafety concerns. She said ethical concerns include that the well-being of all individuals be promoted, that people's value choices and preferences be respected, and that people be treated equitably.

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Dr. Nightingale said the public often feels disenfranchised because of very rapid technological advances. Furthermore, in this area we may be going beyond the ability of the medical profession and biomedical researchers to monitor themselves. She said public participation in decision making is becoming an institutional mechanism for dealing with other complex issues such as environmental hazards and energy concerns. Society has gone beyond relying on the judgement of one person, one discipline, and one profession; oversight committees are becoming necessary.

Dr. Nightingale then discussed the best location for a potential oversight committee. Dr. Nightingale questioned whether the oversight body should be situated in the government. Is a body located in government subject to greater political pressure? If it is outside of government, would it have any impact? She suggested the NIH might not be the best location for an oversight committee; the NIH funds and advocates scientific research. The public might perceive pronouncements coming from an NIH advisory body as favoring science. Moreover, the NIH doesn't deal with other medical issues such as access to health care or the equitable distribution of health care funds.

Dr. Martin thought an oversight group should be as apolitical as possible, and, therefore, probably should not report to a Cabinet officer. He pointed out that RAC, within the NIH, is better isolated from political pressures. Dr. Landy questioned whether political influence on a commission's deliberations is necessarily negative. Dr. Martin expressed concern that special interest groups might have undue and unrepresentative influence on a commission. He noted that our political system is very sensitive and responsive to special interest groups.

Mr. Daloz felt legislation in this area should not be encouraged. He felt the IBCs should be able to provide oversight on most technical matters, while RAC should broaden its scope to include ethical and sociological considerations, and should acquire more members with sociological and industrial backgrounds.

Dr. McKinney asked why social and ethical issues in recombinant DNA, as opposed to other social and ethical issues, have been singled out for consideration. He questioned whether a new commission or review body is required solely to deal with these issues.

Dr. Fedoroff said that RAC has a certain amount of "enforcement power," in that non-compliance with the Guidelines could lead to the loss of Federal research funds. She said she would feel uncomfortable with RAC having "enforcement power" over decisions physicians made in clinical settings.

Dr. Scandalios said committees overseeing human experimentation already exist. Dr. Charles McCarthy, Director of the NIH Office for Protection from Research Risks (OPRR), said the NIH has been delegated responsibility to administer the DHHS regulations for the protection of human subjects. Each institution receiving DHHS funding is required to have an Institutional

Review Board (IRB) to review research involving human subjects; this would include any research that might involve human genetic manipulations. Dr. McCarthy said DHHS is discussing reestablishing an ethics advisory board. Such a board existed from 1978 to 1980 and was advisory to the Secretary, DHEW (now DHHS). If reestablished, that board might review issues such as those discussed in the President's Commission report. Alternatively, RAC might be expanded or some interrelationship between an ethics board and RAC established. Dr. McCarthy added that Senator Kennedy said he would introduce legislation to reestablish a President's Commission.

Dr. Nightingale informed the RAC that the Institute of Medicine of the National Academy of Sciences will be holding a meeting on June 2, 1983, to discuss the need for a new group to replace the President's Commission for the study of Ethical Problems in Medicine and Biomedical and Behavioral Research.

Dr. Nightingale said ethical issues must be discussed in their proper context. In genetic engineering, the context is a technical context; the technical issues must be understood before the ethical issues can be discussed. She said the concept of two separate entities evaluating this issue was, therefore, troubling to her. She suggested that if two separate groups were instituted to provide oversight, these groups might be linked in some way. Dr. Harris concurred with Dr. Nightingale's view. She favored having one group address all the issues; on the other hand, deliberative and regulatory functions are difficult to incorporate into one group.

Dr. Miller of the Food & Drug Administration (FDA) argued that it is not necessary to establish a new regulatory entity. The RAC with its balance of scientific expertise and public representation has admirably met the challenges on the national level. The Institutional Biosafety Committees (IBCs) execute the dictums of the Guidelines at the local level. He pointed out that the IRBs have extensive experience with experimental protocols involving humans, and the ethics attendant to such studies. In addition, an array of regulatory agencies mandated by statute deal with both the process and the products of recombinant DNA experiments. The FDA, for example, will probably regulate the products and process of human gene therapy. Dr. Nightingale asked if Dr. Miller's statement was the official FDA position. Dr. Miller replied that the issue was discussed at the FDA National Center for Drugs and Biologics.

Dr. Martin responded that ethical considerations in genetic engineering will not be limited to human issues. Agricultural and industrial issues will indirectly but quite profoundly affect human society.

Dr. Gottesman felt the major issue facing RAC was to formulate an appropriate response to the President's Commission report. She suggested two possible responses. As the PHS will publish the Commission's report for public comment, RAC members might comment individually. A second possibility is that RAC reply as a group. She suggested an informal poll might gauge RAC's sentiment on these two possibilities. If RAC decides to respond as

a group, a position paper should be prepared for discussion at the September 19, 1983, RAC meeting. Dr. Nightingale suggested the RAC Working Group on Revision of the Guidelines might draft a statement. She said RAC should avoid the appearance of being "self-serving;" if RAC makes a recommendation it should be phrased so it will not be viewed as a way of creating business or perpetuating RAC. Dr. Martin suggested that if RAC were to comment on the President's Commission report, that comment should be published in the Federal Register for public response.

Dr. Gottesman moved that RAC as a group submit a comment on the report, "Splicing Life." The comment would be developed by a RAC working group and presented to the full RAC at the next meeting. In addition, she encouraged RAC members to respond as individuals. She said any response should include, but not be limited to, the following issues:

- (1) Should there be a second oversight group in addition to RAC, or should the job of RAC and an ethical oversight committee be combined? Can RAC alone adequately fill these functions?
- (2) What should be the proportion of scientists to nonscientists on these bodies?
- (3) How does one define the field to be covered by the groups? RAC has a charter; is that charter adequate or inadequate, i.e., if RAC were to cover ethical issues, would the charter have to be changed?
- (4) To whom would the oversight group(s) report?
- (5) If RAC and an ethical oversight group were combined, how would the issue of enforcement or penalties be handled? Is there a difference between decisions on technical and ethical matters?

The working group should attempt to compose a position statement for RAC's consideration. The working group may develop a consensus; but if it does not, it should outline alternatives.

Mr. Mitchell seconded the motion.

Dr. Nightingale said the working group might discuss the advantages and disadvantages of having an array of groups taking care of parts of the problem versus one oversight group discussing the entire gamut of issues. She suggested that information should be assembled on mechanisms already in place to handle parts of the problem.

Dr. Berns called for a vote on Dr. Gottesman's motion. By a vote of nineteen in favor, none opposed, and one abstention, Dr. Gottesman's motion to form a working group to formulate a response to the President's Commission report was approved.

IV. PROPOSED UPDATE OF PROGRAM TO ASSESS RISKS OF RECOMBINANT DNA RESEARCH

When the revised Guidelines for the conduct of recombinant DNA research were issued in December 1978, the Secretary, DHEW (now DHHS), requested that the NIH prepare an NIH Risk Assessment Plan which, after publication in the Federal Register for comment and review by the RAC, would be made final and updated annually. This present document (tabs 1093, 1094, 1106) is the second proposed update. Dr. McGarrity said the objective of the annual update was to review information relevant to recombinant DNA risk assessment.

Dr. McGarrity said he supported the concept of an annual update and thought it should be continued in the future. Drs. Maas, Levine, and Fedoroff agreed. Dr. McGarrity asked whether data from the agricultural area might also be evaluated and incorporated into the annual update.

Dr. McGarrity, noting a discussion of Dr. Freter's observations in Section II-D, Mechanisms That Control Human and Animal Gut Flora, questioned whether plasmid acquisition could in some instances lead to an increased bacterial growth rate rather than the reduced growth rates observed. Dr. Levine said plasmids can apparently either enhance or decrease colonizability and survivability. He said some studies reported in the update are attempts to elucidate the basic mechanisms involved in these processes. He thought many of the answers show there are minimal risks associated with recombinant DNA research. In reading the update, he was impressed with how much progress there has been in the last few years in answering basic questions on colonizability and the effect of plasmids.

Dr. Holmes pointed out that specific parameters in individual risk assessment experiments are limited; however, the complexity of variables affecting experiments is enormous. As an example, Dr. Holmes said he drew conclusions different from those reached by Dr. Levine in Section II-B, Transmission of Vectors from E. coli K-12 to Other Bacteria in Vivo. He thought these experiments show the effect of tetracycline on plasmid transmissibility.

Dr. Levine said several letters commenting on the update had been received by ORDA. While most reiterate the concept that risks are minimal, the letter from EPA points out the need for risk assessment with respect to intentional release into the environment. Dr. Fedoroff agreed and said that when RAC reviews cases of requests for release to the environment of genetically engineered organisms, the investigator should be asked to include monitoring of the dissemination of the organisms.

Dr. Gottesman pointed out that risk assessment experiments with E. coli K-12 were relevant when they were designed and performed. Now the Guidelines permit many other types of organisms to be used, so K-12 risk assessment data are less relevant. She said designing a general risk assessment protocol is difficult. Dr. Gottesman thought a more appropriate approach might be to ask investigators to add risk assessment to specific experiments they are doing.

Dr. Levin of the EPA said his agency was examining some aspects of deliberate release of microorganisms into the environment. These data will be shared with the RAC. He pointed out that interpreting these experiments requires expertise in population genetics and population biology. He said questions such as the following have to be considered: (1) what happens if genetic drift occurs; and (2) what happens if in the process of altering a plasmid, the rate at which it is transmitted is changed? Dr. Levin pointed out that the outcome of the introduction of an organism with a novel genotype into the environment cannot be predicted.

Dr. Fedoroff said she supported the letter from Dr. Gill (tab 1094) stating that the experiments proposed by Dr. Murphy, Cloning and Expression of DNA Coding for Diphtheria Toxin, should not be included in the annual update as Section II-G. These experiments would not provide general risk assessment information. Dr. Holmes agreed. Dr. Fedoroff moved that Section II-G be deleted from the proposed update. Dr. Holmes seconded Dr. Fedoroff's motion. He said RAC's approval for the experiment to proceed at P4 containment, as was given to Dr. Murphy, did not reflect RAC support of the experimental goal; rather it indicated RAC's judgment that P4 could safely contain the experiment. He thought the use of the words "on the recommendation of the RAC" in the draft risk assessment plan gave the erroneous appearance that RAC was encouraging the work. Dr. Talbot said that Dr. Murphy's proposal will be reviewed for scientific merit by an NIH study section. A decision will then be made on whether or not to allow the experiment to proceed in the NIH P4 facility.

By a vote of thirteen in favor, one opposed, and six abstentions, the RAC recommended Section II-G be deleted from the second annual update.

V. CDC-NIH GUIDELINES AND NCI REVISION OF ONCOGENIC VIRUS GUIDELINES

Dr. Barkley, Director of the NIH Division of Safety and Chairman of the RAC Working Group on Classification of Microorganisms, reported on the revision of the CDC (Centers for Disease Control) - NIH guide to microorganisms, entitled "Biosafety in Microbiological and Biomedical Laboratories" which will be distributed for comment. The guide covers pathogens which: (1) are documented hazards to laboratory personnel, (2) pose a high potential risk to laboratory personnel, or (3) may produce diseases of grave consequence should infection occur. Dr. Barkley said the document also refines the four classes of safeguards, P1 through P4, that were first developed for the NIH Guidelines for Research Involving Recombinant DNA Molecules. Dr. Berns said this version represents a major effort by Dr. Barkley and collaborators who have done a terrific job. Dr. Barkley said that after reviewing the comments received on the draft, it is hoped that a final document will be ready soon after September 1, 1983.

Dr. Barkley then reported on the status of revision of the National Cancer Institute (NCI) Safety Standards for Research Involving Oncogenic Viruses. These standards, issued in 1974, specify three levels of control: low, moderate, and high. The high level, equivalent to P4 containment, is

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reserved for proven human cancer viruses. The moderate level is approximately equivalent to P3 containment, and the low level is approximately equivalent to the P2 containment level. He said a committee had been formed within NCI to reassess the standards in light of information gained over the last decade. The committee also is attempting to recommend safeguards which correspond to those specified in the CDC-NIH Guidelines. Dr. Barkley said the NCI revision is expected to be completed in June.

Dr. Barkley said the RAC Working Group on Classification of Microorganisms will use the revised NCI viral oncology standards and the CDC-NIH Guidelines to make recommendations for revision of Appendix B, Classification of Microorganisms on the Basis of Hazard, of the NIH Guidelines for Research Involving Recombinant DNA Molecules.

VI. REPORT OF WORKING GROUP ON REVISION OF THE GUIDELINES

Dr. Nightingale reported on the January 21, 1983, meeting (tab 1102) of the Working Group on Revision of the Guidelines. She said the working group discussed several topics:

- (1) agricultural issues, particularly dissemination into the environment for plants;
- (2) a review of the letters received from Institutional Biosafety Committee (IBC) chairpersons in response to a questionnaire on IBC function;
- (3) the desirability of expediting reviews of proposals between RAC meetings and if so, how;
- (4) a proposal to incorporate the Physical Containment Recommendations for Large-Scale Uses of Organisms containing Recombinant DNA Molecules into the Guidelines as an appendix;
- (5) the status of Recombinant DNA Advisory Committee (RAC) subcommittees and working groups; and
- (6) the current requirements for P4 physical containment.

Dr. Nightingale said the working group first discussed the issue of field experimentation with plants. The working group agreed that language specifying guidelines for agricultural field experimentation should be developed. Dr. Nightingale said Drs. Tolin and Scandalios were assigned this task. The language developed by Drs. Tolin and Scandalios will be discussed later later in the RAC meeting. (See Item VIII. Proposed Amendment of Section III-A-2 and Addition of a New Section III-B-4-c.)

Dr. Nightingale said the working group then reviewed the responses of the IBCs (tab 1099) to a questionnaire. Dr. Nightingale said ORDA had solicited responses from IBC chairpersons concerning:

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- o problems with the Guidelines;
- o what things are taking large amounts of time;
- o what things are taking inappropriate amounts of time;
- o in what areas do IBCs disagree with the RAC with regard to containment for a particular experiment;
- o in what ways are the Guidelines too stringent or too relaxed;
- o how frequently does the IBC meet; and
- o does the IBC have other responsibilities at the institution.

She said approximately 250 questionnaires were mailed. ORDA received 45 responses, a low response, but probably an indication of an absence of problems at the IBC level. Dr. Nightingale said few of the respondees feel overburdened. Most IBCs deal only with recombinant DNA issues. Those feeling they have a heavy workload (such as the Harvard University IBC) do not wish to assume any additional biosafety tasks. Other IBCs feel they could expand.

Dr. Nightingale said the Schering Corporation IBC suggested that a system be implemented by RAC and/or ORDA to audit IBC functions to ensure that they are operating in accordance with the Guidelines.

The IBC of the State University of New York at Albany requested that all experiments, including exempt experiments, be registered; the IBC argued that it is impossible otherwise to know if the decision by the principal investigator that his experiment was exempt was correct.

Some IBCs wrote it would be helpful to have "a guide to the Guidelines" even though they felt the Guidelines were now easier to understand, easier to follow, and on the whole quite satisfactory. This guide might be a subject index, an investigator use packet, or an expanded table of contents.

Dr. Nightingale said some letters from IBC chairpersons suggested clarifications in the Guidelines. For example, Section III-C should be clarified to specify when registration documents are to be filed. While the title of Section III-C indicates that notice must be filed simultaneously with initiation of experiments, the text does not. Dr. Nightingale moved that the language of the first paragraph of Section III-C be amended to indicate when the registration document should be filed with the IBC. By a vote of twenty in favor, none opposed, and one abstention, the RAC recommended that the language of Section III-C be clarified at the next printing of the Guidelines.

Dr. Nightingale said respondees urged that the listing of low and moderate risk oncogenic viruses in Sections B-II-A and B-II-B of Appendix B be clarified. These viruses are not classified with a particular risk specification as are other agents in the Appendix. To further confuse matters, these viruses are listed in the text between Class 4 and Class 5 agents. Dr. Nightingale expressed hope that the working group chaired by Dr. Barkley would resolve this issue.

In summary, Dr. Nightingale said the comments from IBCs on the whole were very supportive of the Guidelines, and there were no major problems.

Dr. Nightingale said the Working Group on Revision of the Guidelines then discussed the desirability of expediting reviews of proposals received between RAC meetings, as the period of time elapsing between RAC meetings is increasing. The working group concluded, after some discussion, that the items that come to the full RAC for evaluation are important and complex; and, therefore, the current procedures should be retained for the time being.

Dr. Nightingale said the working group discussed the proposal, to be evaluated by RAC later in the meeting, to incorporate the Physical Containment Recommendations for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules into the Guidelines. (See Item XIV. Proposed Incorporation into Guidelines of Physical Containment Recommendations for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules.) The working group agreed that the Recommendations should be incorporated into the Guidelines.

Dr. Nightingale said the working group then discussed the status of the RAC subcommittees. Dr. Nightingale said the RAC charter stipulates three subcommittees: the Risk Assessment Subcommittee, the Host-Phage Subcommittee, and the Host-Plasmid Subcommittee. The subcommittees are standing committees authorized in the charter. Working groups, on the other hand, are not provided for in the charter but can be created by the RAC to serve a specific function and dissolved when no longer necessary.

Dr. Nightingale noted that two of the three subcommittees, the Host-Phage and the Host-Plasmid Subcommittees, have not met for some time, and no issues will be placed before them in the foreseeable future. The Working Group on Revision of the Guidelines suggested that the process to revise the RAC charter to delete these two subcommittees be initiated; and also that two of the current working groups, the Working Group on Revision of the Guidelines and the Plant Working Group, be changed to subcommittees. Dr. Nightingale asked if the RAC would have to vote on this suggestion. Dr. Talbot replied that no vote was necessary and that if no RAC members objected (and none did), NIH staff would proceed to request the charter changes. These changes would require final action by the Secretary of the Department of Health and Human Services.

Dr. Nightingale said the working group also discussed the P4 physical containment specifications. That topic will be discussed later in the RAC meeting. (See Item VII. Proposed Modification of P4 Containment.)

VII. PROPOSED MODIFICATION OF P4 CONTAINMENT

Dr. McKinney began the discussion on modifying the requirements of P4 containment (tabs 1102, 1107, 1101/I). Dr. McKinney said this topic had been broached at the January 21, 1983, meeting of the Working Group on Revision of the Guidelines.

At that meeting, Dr. Malcolm Martin of the NIH suggested Appendix G-II-D-2-a of the Guidelines be modified. That section specifies that:

"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."

Dr. Martin said the specification requiring use of the Class III glove box is meant to protect the investigator against aerosol contamination. He said the Class III glove box does not, however, afford protection when infection by the organism being studied does not occur by aerosol exposure. He argued that automatic assignment of experiments to the glove box ties up the staff of the P4 facility, since all manipulations are more difficult to perform in the glove box. Dr. Martin suggested the language of Appendix G-II-D-2-a be amended to include a statement that:

"...in those situations where an aerosol will not be generated or when illness is not caused by aerosol exposure, the research must be conducted in the P4 facility, but options for working outside the glove box may be available."

The Working Group on Revision of the Guidelines agreed language providing greater flexibility in use of the P4 facility should appear in the Federal Register as a proposed action for a period of comment. After consultation, NIH staff determined that Appendix G-II-D-2-c might more appropriately be modified. The following proposed modification was published in the Federal Register:

"Appendix G-II-D-2-c. 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 can be conducted in the P4 facility using containment equipment requirements specified for the P3 level of containment. Alternative combinations of containment safeguards are shown in Table II. In those cases where the host is an organism which does not cause infection by the respiratory route (e.g., use of E. coli K-12 or S. cerevisiae host-vector systems), the local IBC may set appropriate containment for procedures within the P4 facility."

Dr. McKinney said he did not agree with the rationale offered for the suggested modification to Appendix G-II-D-2-c. He said the proposed language was unclear and did not reflect the need to operate the P4 facility according to standard practices. He suggested (tab 1107) the following wording be substituted:

"In certain circumstances the nature of an experiment may dictate the use of a P4 facility without the requirement to employ a host-vector system that provides a one-step higher level of biological containment. In these cases, the local IBC may approve the experiment to be conducted using the practices and primary containment equipment specified for the P3 level of physical containment. Election of either of the alternatives defined in this Appendix does not alter the requirement to comply with the other practices, procedures, and operational conditions defined for the P4 level of physical containment."

Dr. McGarrity said the rationale for modifying this section is not convincing, and does not describe the capabilities of a Class III glove box. He added that the proposed modifications do not address Appendix G-II-D-1-j, which states that material within the Class III cabinet shall be removed from the cabinet system only after being steam sterilized or contained in a nonbreakable sealed container. He said he could not support either the proposal published in the Federal Register or the alternative proposed by Dr. McKinney.

No RAC member offered a motion concerning the proposal, and the discussion ended.

VIII. PROPOSED AMENDMENT OF SECTION III-A-2 AND ADDITION OF A NEW SECTION III-B-4-c

Dr. Tolin introduced Dr. Anne Vidaver of the University of Nebraska, an ad hoc consultant to the RAC on agricultural issues. Dr. Vidaver reviewed the proposal (tabs 1100, 1101/VII) to amend Section III-A-2 and add a new Section III-B-4-c to the Guidelines. The RAC Working Group on Revision of the Guidelines at its January 21, 1983, meeting, recommended that guidelines for field testing of plants modified by recombinant DNA be developed for consideration at the April 11, 1983, RAC meeting. In response to that mandate, the following changes in the Guidelines were proposed and published for comment in the Federal Register (48 FR 9441):

"Section III-A-2 would be modified to read as follows:

"III-A-2. Deliberate release into the environment of any organism containing recombinant DNA except certain plants as described in Section III-B-4-c.

"A new Section, III-B-4-c, would be added as follows:

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"III-B-4-c. Approval may be granted by the IBC with notification to ORDA for growing plants containing recombinant DNA in the field under the following guidelines:

"III-B-4-c-(1). The plant species is an annual cultivated crop of a genus that has no species known to be a noxious weed.

"III-B-4-c-(2). The introduced DNA consists of well characterized genes containing no sequences harmful to humans, animals, or plants. Antibiotic resistance genes may be introduced as selectable marker traits if stable integration into the host DNA is known to occur.

"III-B-4-c-(3). The vector consists of DNA from (i) exempt host-vector systems (Appendix C); (ii) plants of the same or closely related species; (iii) non-pathogenic prokaryotes or nonpathogenic lower eukaryotic plants; (iv) plant pathogens if known sequences causing disease symptoms have been deleted; or (v) DNA constructed from specific sequences of any of the above sources.

"The DNA may be introduced by any suitable method but if co-infection or co-cultivation is utilized absence of the assisting organism must be demonstrated.

"III-B-4-c-(4). Plants are grown in control access fields under specified conditions appropriate for the plant under study in the geographical location. Such conditions should include provisions for using good cultural and pest control practices, for physical isolation from plants of the same species outside of the experimental plot in accordance with pollination characteristics of the species, and for preventing plants containing recombinant DNA from becoming established in the environment. Review of the IBC should include an appraisal by scientists knowledgeable in the crop, its production practices, and the local geographic conditions."

Dr. Tolin said the proposed language does not provide blanket approval for field testing plants. Rather review responsibilities are shifted from the RAC to the local IBC when field tests meet certain defined criteria. ORDA would be notified before initiation of the experiments.

Dr. Vidaver commended Drs. Tolin and Scandalios for preparing the proposed language. She suggested the word "annual" be deleted from proposed Section III-B-4-c-(1). Dr. Tolin said the intent was to limit exposure in the field to a single season. She agreed the word "annual" should be deleted.

Dr. Scandalios suggested the language of proposed Section III-B-4-c-(3) should be modified to read in part:

"...or (v) chimeric vectors constructed from sequences defined in (i) to (iv) above. The DNA may be introduced by any suitable method."

The remainder of the proposed sentence, which reads as follows, would be eliminated:

"...but if co-infection or co-cultivation is utilized absence of the assisting organism must be demonstrated."

Dr. Fedoroff proposed that the language of proposed Section III-B-4-c-(2) be modified to read in part:

"...Antibiotic resistance genes may be introduced as selectable marker traits if stable integration into the host DNA is known to occur, and the antibiotic is one not generally used for treatment of human, animal, or plant diseases."

Dr. Vidaver asked if Section III-A-3 of the Guidelines prohibits the deliberate transfer of a drug resistance trait. Dr. Nightingale said Section III-A-3 refers to microorganisms and does not cover plants.

Dr. Brill said restriction of the use of antibiotic markers will stifle plant molecular biology. He said selectable markers, whose products don't kill or debilitate the plant or prevent regeneration, are necessary and the selectable markers many laboratories use are antibiotic resistance genes.

Dr. Tolin said the issue of the use of antibiotic markers had been discussed at the January 21 meeting of the Working Group on Revision of the Guidelines. From the discussion, Dr. Tolin reasoned that if a gene is stably integrated into the chromosome, the probability that it might be transferred to other organisms is extremely low. Dr. Gottesman said stable integration of some of the DNA does not mean other copies could not be transferred to other organisms, only that a mechanism for stable integration exists.

Dr. Vidaver said that a large percentage of microorganisms found in nature already harbor multiple antibiotic resistance markers. Some of these antibiotics are clinically important. She saw no justification for restricting the use of genes coding for antibiotic resistance in plants. Dr. Clowes said he did not understand the proposed restriction on the use of antibiotic resistance markers in plants. He could understand why one would not want such resistance to get into microbial pathogens for which the antibiotic is used in treatment. The case in plants, however, was completely different. Dr. Fedoroff withdrew her proposed amendment.

Dr. Gottesman said the proposed Federal Register language does not specify containment. Dr. Fedoroff added that there is no data base for these types of experiments. She suggested these experiments could generate some risk assessment data on dispersal of organisms containing recombinant DNA. She proposed that the following sentence be added at the end of Section III-B-4-c-(4):

"Procedures for assessing the spread of organisms containing recombinant DNA must be developed and approved by the IBC and the results of the test should be submitted for review to the IBC and copies submitted to the Risk Assessment Subcommittee of the RAC."

Dr. Fedoroff said in these experiments, the assumption is that the DNA will spread.

Dr. McKinney suggested the language proposed by Dr. Fedoroff be clarified. He added that proposed Section III-B-4-c-(4) mentions provisions for "physical isolation" of plants. He said physical isolation cannot be obtained under the described conditions and suggested that term not be employed.

Dr. McKinney questioned why "species" are mentioned in Section III-B-4-c-(4) while the term "genus" is used in Section III-B-4-c-(1). Dr. Tobin said "species" is used in Section III-B-4-c-(4) to indicate physical isolation from other commercial plants of the same species. "Genus" is used in Section III-B-4-c-(1) because intraspecies hybridization occurs in some genera.

Dr. McKinney said Dr. Vidaver had proposed that the word "annual" be deleted from Section III-B-4-c-(1), while Section III-B-4-c-(4) includes a provision for preventing plants containing recombinant DNA from "becoming established" in the environment. He found this incongruous as he intuited dissemination increases with time. He questioned the definition of "established in the environment;" after what period of time is a plant "established" in the environment?

Dr. McKinney questioned whether risk assessment on the possible development of noxious weeds could be performed. He noted that attempts would be made to engineer plants that will grow faster, better, in poorer soil, or with less fertilizer. These are characteristics of "weeds."

Dr. Landy said Dr. Fedoroff's proposed amendment would provide information but questioned its usefulness for risk assessment. Dr. Holmes said spread is inevitable and questioned the usefulness of monitoring.

Dr. Brill said quantitative risk assessment experiments can not be performed at this stage since no one has a good idea of any kind of real risk. He said most successful plant breeding is performed through observation rather than quantitation. He suggested the observational powers and experience of breeders might be used in risk assessment. He recommended the IBC perform risk assessment by having one or more individuals familiar with the crop observe the crop, the neighboring crop, the field, and the surrounding fields during the growing season.

Dr. Fedoroff said she still favored addition of a sentence to be added at the end of Section III-B-4-c-(4) although she changed the first words from "Procedures for assessing the spread..." to "Procedures for assessing alterations in and the spread...."

Dr. Gottesman said she was uncomfortable with the prospect of full responsibility being delegated to the IBC. She moved that review of these experiments be performed by the RAC Plant Working Group and that the proposed language be incorporated into the Guidelines as an appendix.

As part of her motion, she proposed the following language in Section III-B-4-c-(2) be deleted:

"Antibiotic resistance genes may be introduced as selective marker traits if stable integration into the host DNA is known to occur."

She also accepted in the motion the language proposed by Dr. Fedoroff concerning risk assessment to be added to Section III-B-4-c-(4). Some additional language modification and the proposed modifications in Sections III-B-4-c-(1) and III-B-4-c-(3) would be part of the motion. The motion was seconded.

Dr. Nightingale felt that RAC should not delegate its authority to a working group. Dr. Fedoroff agreed. She then offered a substitute motion to incorporate the language of Dr. Gottesman's motion into the Guidelines. However, only IBC review and approval would be required, as in the original Tolin-Scandalios proposal, with no review by the RAC Plant Working Group. Her amendment to require risk assessment language to be included in Section III-B-4-c-(4) would be part of the substitute motion. The substitute motion was seconded.

Dr. Gottesman felt too much responsibility would be assigned to the IBCs if Dr. Fedoroff's substitute motion were accepted. Review by a RAC working group would place responsibility midway between RAC and the IBCs. She pointed out that proposals involving the cloning of genes for certain toxins potent for vertebrates are reviewed currently by a mechanism similar to her proposal.

A motion to call the question passed by a vote of eighteen in favor, none opposed, and one abstention.

By a vote of eight in favor, eleven opposed, and one abstention, Dr. Fedoroff's substitute motion was refused. Dr. Brill abstained.

The vote was then called on Dr. Gottesman's motion to permit the Plant Working Group to grant approval for field testing plants containing recombinant DNA under the following conditions:

- "(1) The plant species is a cultivated crop of a genus that has no species known to be a noxious weed.
- "(2) The introduced DNA consists of well characterized genes containing no sequences harmful to humans, animals, or plants.
- "(3) The vector consists of DNA (i) from exempt host-vector systems (Appendix C); (ii) from plants of the same or closely related species; (iii) from nonpathogenic prokaryotes or nonpathogenic lower eukaryotic plants; (iv) from plant pathogens if known sequences causing disease symptoms have been deleted; or (v) from chimeric vectors constructed from sequences defined in (i) to (iv) above. The DNA may be introduced by any suitable method.

- "(4) Plants are grown in control access fields under specified conditions appropriate for the plant under study and the geographical location. Such conditions should include provisions for using good cultural and pest control practices, for physical isolation from plants of the same species outside of experimental plot in accordance with pollination characteristics of the species, and for further preventing plants containing recombinant DNA from becoming established in the environment. Review by the IBC should include an appraisal by scientists knowledgeable of the crop, its production practices, and the local geographic conditions. Procedures for assessing alterations in and the spread of organisms containing recombinant DNA must be developed. The results of the outlined tests must be submitted to and reviewed by the IBC. Copies must also be submitted to the Plant Working Group."

The language would be incorporated into the Guidelines as an appendix. By a vote of twelve in favor, six opposed, and two abstentions, the motion was accepted. Dr. Brill was one of the abstainers.

Dr. Gottesman then moved to modify the language of her motion. She proposed deleting item (iv) in Section III-B-4-c-(3).

Dr. Vidaver pointed out that deleting item (iv) would restrict research with the Ti plasmid, one of the major mechanisms for introducing DNA into plants. Dr. Martin suggested the word "known" could be deleted.

Drs. Gottesman, Tolin, and Vidaver then developed the following alternative language for item (iv) in Section III-B-4-c-(3):

"(iv) from plant pathogens only if the sequences causing disease have been deleted."

Dr. Gottesman substituted this language as her proposed motion. Dr. Harvin seconded the motion.

Dr. Martin thought the phrase "use of non-pathogenic portions or fractions of sequences" was more specific than the phrase "disease causing sequences." Dr. Tolin said she objected to Dr. Martin's proposed language because it would restrict plant research with viral vectors.

Dr. Landy asked if Dr. Gottesman's proposed language implies all genes necessary to cause disease be deleted or if only one of these genes need be deleted. Dr. Fedoroff said only one gene need be removed. Dr. Martin said the language is intended to permit use of portions of potentially pathogenic organisms or DNAs as vectors; however, only portions which do not cause disease are to be used.

Dr. Berns called the vote on the motion to change the language of item (iv). By a vote of eighteen in favor, none opposed, and one abstention, the RAC noted to amend Section III B-4-c-(3). Dr. Brill abstained.

IX. REQUEST TO RELEASE STRAINS OF PSEUDOMONAS SYRINGAE AND ERWINIA HERBICOLA

Dr. Berns called upon Dr. Vidaver to review the proposal (tabs 1103, 1101/VI) to field test genetically modified strains of Pseudomonas syringae and Erwinia herbicola. The proposal, submitted by Drs. Nickolas Panopolous and Steven Lindow of the University of California, Berkeley, requests permission to field test these organisms to control, biologically, frost damage in plants. The strains would be carrying deletions of all or part of the genes involved in ice nucleation.

When freezing occurs on plant tissues, the tissues are damaged on thawing. Bacteria then enter through the damaged tissue and destroy the plant or at least destroy the tissue. Certain bacteria, such as Pseudomonas syringae (various pathovars under the current taxonomic classification), Erwinia herbicola, and, occasionally, isolates of Pseudomonas fluorescens, serve as nuclei for ice crystal formation in supercooled water at temperatures slightly below 0°C (threshold nucleation temperature about -1.8°C). These bacteria are common plant epiphytes found in substantial numbers on above-ground plant surfaces (leaves, twigs, buds, flowers) with seasonal fluctuations from undetectable levels up to 10⁷ cells/gram tissue fresh weight. A causal relationship between frost damage on frost sensitive crop plants at relatively warm subzero temperatures (down to -5°C) and these organisms has been established. For instance, the degree of damage after exposure to low temperatures either in the field or in controlled environments (growth chambers) is directly related to the populations of ice nucleation active (INA⁺) bacteria present in or on the surfaces of above ground parts. Plants grown aseptically can tolerate temperatures of several (ca. 6) degrees Celsius below zero without apparent damage. They are rendered sensitive to such temperatures by spraying with suspensions of INA⁺ bacteria prior to low temperature exposure. Frost damage to plants can be decreased by reducing the natural epiphytic population of INA⁺ bacteria; significant protection against frost damage has been obtained by application of various bactericides. The use of antagonistic bacterial strains which compete with the natural epiphytic flora has also been shown to be effective under field conditions. INA⁻ mutants of the naturally occurring INA⁺ strains should be especially suitable antagonists. These mutants, being basically adapted for epiphytic growth and survival, presumably displace their wild-type counterparts by occupying the same physical spaces and utilizing the same nutrients.

Dr. Vidaver noted this request had been previously reviewed by the RAC at its October 25, 1982, meeting. She said RAC had recommended approval of the proposal by a narrow margin (seven in favor, five opposed, and two

abstentions), and that several questions were raised during the discussion. Because of concerns raised at the RAC meeting, approval of the proposed field tests was withheld by NIH.

Dr. Vidaver said the investigators, in their current proposal, addressed the issues raised at the October 25, 1982, meeting. She said the investigators have modified their proposal to test only in one location rather than in the six locations proposed in the original request. The investigators have also addressed questions of construction, handling, safety, and testing and are asking RAC to comment on the choice of antibiotic resistance markers.

Dr. Vidaver thought that the proposed use of bactericides as part of the proposed emergency plan could not be recommended. She said burning or burying were preferable procedures.

Dr. Vidaver said the investigators have presented several arguments in support of their proposal. These are:

- (1) P. syringae pv syringae and E. herbicola are common and omnipresent plant epiphytes. P. syringae strains have also been widely applied to plants as a biological control agent of certain plant diseases.
- (2) Ice nucleation activity, in addition to causing frost damage, is considered to be a conditional frost-dependent virulence factor in P. syringae pv. syringae. The INA⁻ deletion mutants can be considered at least partially "debilitated" with respect to virulence.
- (3) E. herbicola is not pathogenic on plants (with rare exceptions, on crops not grown in California).
- (4) Although certain strains of P. syringae can be pathogenic to hosts such as pear, almond, and citrus, disease caused by this pathogen on these hosts is rare in California and occurs only after predisposition by freezing injury. All P. syringae strains used in the experiments will be isolated from the surfaces of healthy plants, not from disease lesions. The use of avirulent non-toxin producing strains, in locations where susceptible crops are absent, on crops non-susceptible to the bacterium in California's climate will protect crop plants of the region. As P. syringae is ubiquitous the likelihood of increased disease on either homologous or heterologous plants appears remote.
- (5) The habitats of P. syringae and E. herbicola differ both in space and in time although they partially overlap; the epiphytic population cycle of E. herbicola during the growing season differs from that of P. syringae p.v. syringae.

- (6) Studies with genetically marked strains have shown that the bacteria do not survive well through a full yearly cycle, i.e., their populations drop to very low levels during the dry season. These epiphytic bacteria do not survive for more than a month in the soil. The bacteria can survive up to a year in plant debris on the surface of the soil, but die rapidly when plant debris is incorporated into the soil. All plant debris in trial locations will be promptly incorporated into the soil upon completion of each experiment. Although some movement of bacteria to aerial sites near treatment locations by insect or aerial dispersal is possible, the numbers of viable cells transported has been shown to be very small; and these cells are subject to biological and physical processes limiting survival.
- (7) Wild-type strains of both bacteria have been sprayed on the field over several years with no adverse effects on humans. P. syringae p.v. syringae is not a human pathogen; indeed, it does not grow at temperatures above 33-34°C. Pathogenicity of E. herbicola to humans appears to be a property of E. herbicola strains of human origin but not of plant origin. The strains to be used in this study were isolated from healthy plants.
- (8) With the exception of streptomycin and tetracycline, antibiotics are not commonly used for plant disease control in temperate regions. Use of resistance markers to these antibiotics will be specifically avoided.
- (9) Growth chamber and greenhouse experiments will be performed to ascertain that the engineered strains do not cause frost injury to plants.
- (10) The impact of INA⁻ deletion mutants on rainfall patterns is thought to be extremely small or non-existent. The extremely small scale of these trials compared to the amount of agricultural and natural vegetation suggest that any potential reductions of atmospheric ice nuclei would be negligible. There are no reports of alterations in rainfall patterns following large-scale use of agricultural bactericides over the last four decades. The microbiological impact of field trials on the natural epiphytic populations of ice nucleating bacteria would be negligible compared to that caused by standard agronomic practices such as orchard pruning or crop selection by farmers.

Dr. Vidaver said that "worst case" experiments have already been done, i.e., these organisms modified by classical genetic techniques have already been released. In addition, she said that the populations to be released are 8 to 10 orders of magnitude lower than those normally found in the environment.

Dr. Tolin felt the investigators had satisfactorily addressed the issues raised at the October 25, 1982, RAC meeting. Dr. Fedoroff said she was satisfied with the proposal. She said the investigators have admirably

designed their experiments to assess the ability of the altered organisms to compete with resident organisms. She said she would like to see these data when they become available.

Dr. Holmes responded to the request to evaluate the antibiotic resistance genes proposed for use as markers. He said use of some of the proposed antibiotic markers raises minimal concern. However, he thought rifampicin resistance is not widely disseminated in nature and is useful in treating certain human diseases. For this reason he suggested rifampicin not be used. Dr. Friedman said rifampicin resistance is encoded by a chromosomal gene, so resistance probably would not be transferred to other organisms.

Dr. Martin moved acceptance of the proposal with exclusion of the use of antibiotics for emergency procedures. By a vote of nineteen in favor, none opposed, and no abstentions, the motion was recommended.

X. PROPOSED MODIFICATIONS OF SECTION III-B-5 OF THE GUIDELINES

Dr. McKinney said the RAC Large-Scale Review Working Group, which met on October 26, 1982, has forwarded to RAC without comment a proposal (tabs 1095/I/A, 1101/II) by Dr. Allan Waitz of DNAX Corporation (a wholly owned subsidiary of Schering-Plough Corporation) to amend Section III-B-5 of the NIH Guidelines. Section III-B-5 specifies that for experiments involving more than 10 liters of culture appropriate containment will be determined by the IBC. Dr. Waitz argued that greater flexibility in scale-up procedures would be gained if the IBC could be notified simultaneously with initiation of large-scale procedures involving E. coli K-12, Saccharomyces cerevisiae and Bacillus subtilis host-vector systems. Dr. Waitz suggested that Section III-B-5 be modified to read as follows:

"III-B-5. Experiments Involving More than 10 Liters of Culture. The appropriate containment will be decided by the IBC except where exempted under Section III-D-5. Where appropriate, the large-scale containment recommendations of the NIH should be used (45 FR 24968)."

Language in Appendix C, Exemptions Under III-D-5, would also be modified to reflect this change. The relevant paragraphs of Appendix C-II, C-III, and C-IV, which deal with exceptions to the exemption, would be modified to read as follows:

"Large-scale experiments (i.e., more than 10 liters of culture) require IBC notice simultaneously with the initiation of experiments where IBC-approved practices and an IBC-approved P1-LS containment facility will be used. Where these conditions are not satisfied, refer to Part III-B-5."

Mr. William Muth of Eli Lilly and Company said approval of the modification would permit companies greater flexibility. Dr. Gottesman said she would vote against the motion. She thought it appropriate that the IBC review the proposal before large-scale procedures were initiated. She felt the company would know in advance of plans to scale-up, and the company and the IBC could prepare accordingly.

Dr. McKinney moved approval of the proposal. Dr. McGarrity seconded the motion.

By a vote of ten in favor, eight opposed, and one abstention, the motion was accepted. Dr. Martin abstained.

XI. PROPOSED MODIFICATION OF SECTION VII-D-6 OF THE PHYSICAL CONTAINMENT RECOMMENDATIONS

Dr. McKinney presented the proposal (tabs 1095/II/A, 1101/III/A) to modify Section VII-D-6 of the Physical Containment Recommendations for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules. Dr. McKinney said Mr. Richard F. Geoghegan of E. I. DuPont de Nemours and Company requested that Section VII-D-6 of the Large-Scale Recommendations be revised. Section VII-D-6 specifies that:

"A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be operated so that the space above the culture level will be maintained at or slightly below atmospheric pressure."

Mr. Geoghegan proposed that Section VII-D-6 be modified to specify that the "closed system ... shall be operated so that the space above the culture level be maintained at no more than 10 psig."

Mr. Geoghegan argued that the revision will permit fermentations more in line with industrial practices (high biomass production through efficient oxygen transfer) to be conducted without compromising safety by operating at unnecessarily high vessel pressures. The RAC Large-Scale Review Working Group, at its meeting on October 26, 1982, felt the language might appropriately be modified, but felt no specific pressure limit should be indicated as the pressure limit should be dependent on the maximum design pressure of the system. The working group, thus, suggested the following language:

"VII-D-6. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be operated so that the space above the culture level will be maintained at a pressure as low as possible, consistent with equipment design, in order to maintain the integrity of containment features."

The Large-Scale Review Working Group at its meeting on October 26, 1982, recommended this language by a vote of five in favor, none opposed, and no abstentions.

Dr. McKinney moved that the RAC accept the language proposed by the RAC Large-Scale Review Working Group. Dr. Martin seconded the motion.

By a vote of nineteen in favor, none opposed, and no abstentions, the RAC recommended the motion.

XII. PROPOSED MODIFICATION OF SECTIONS VII-B-1, VII-C-1, and VII-D-1 OF THE PHYSICAL CONTAINMENT RECOMMENDATIONS

Dr. McKinney introduced the proposal (tabs 1095/II/B, 1101/III/B, 1104) of Dr. Allan Waitz to revise Sections VII-B-1, VII-C-1 and VII-D-1 of the Physical Containment Recommendations for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules. The current language specifies that cultures of viable organisms containing recombinant DNA molecules shall be handled in closed systems which are designed to reduce the potential for escape (Section VII-B-1) or prevent (Sections VII-C-1 and Section VII-D-1) escape of viable organisms.

Dr. Waitz suggested the first sentence in Sections VII-B-1, VII-C-1, and VII-D-1 should be modified to read as follows:

"Cultures of viable organisms containing recombinant DNA molecules shall be handled in a closed system used for the propagation, growth and processing of cultures, other primary containment equipment, or other appropriate method of containment approved by the IBC, which is designed to reduce the potential for escape of viable organisms...."

The Large-Scale Review Working Group, at its meeting on October 26, 1982, pointed out that a closed primary system is one from which there is no release of organisms into the environment or work place; a primary system could be defined as a fermentor attached to several pieces of processing equipment. The working group agreed that the language as currently written is purposely flexible while at the same time conveying the intent; they, therefore, recommended against accepting this proposal.

Dr. McKinney said he felt the current language is adequate. No motion was offered and the discussion ended.

XIII. PROPOSED MODIFICATION OF SECTION VII-B-3 OF THE PHYSICAL CONTAINMENT GUIDELINES

The proposal (tabs 1095/II/C, 1101/III/C) of Dr. Allan Waitz to modify Section VII-B-3 of the Physical Containment Guidelines for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules was introduced by Dr. McKinney. Section VII-B-3 reads as follows:

"VII-B-3. Sample collection from a closed system, the addition of materials to a closed system and the transfer of culture fluids from one closed system to another shall be done in a manner which prevents the release of aerosols or contamination of exposed surfaces."

Dr. Waitz suggested that the word "minimizes" be substituted for the word "prevents." He suggested that the word "prevents" implies an absolute condition which at the P1-LS level is neither realistic nor necessary. Dr. Waitz said the Schering Corporation IBC interprets "prevents" in an absolute sense. Mr. Muth said the Eli Lilly and Company IBC also tends to be conservative in interpretation. Dr. Harvin pointed out that the word "minimize" is open to a great deal of interpretation.

Dr. McKinney said the Large-Scale Review Working Group did not discuss this proposal at its meeting on October 26, 1982. Dr. McKinney said he was not prepared to discuss the difference between the word "prevents" and the word "minimizes." He thought the word "prevents" is permissive, and suggested the current wording is adequate.

Dr. Gottesman asked if procedures would change if the word "minimizes" was substituted for "prevents." Dr. Waitz said he did not believe procedures would be markedly different.

Dr. Martin moved that the word "minimize" be substituted for the word "prevents" in Section VII-B-3. Dr. Daloz seconded the motion.

By a vote of ten in favor, four opposed, and four abstentions, the RAC recommended the motion.

XIV. PROPOSED INCORPORATION INTO GUIDELINES OF PHYSICAL CONTAINMENT RECOMMENDATIONS FOR LARGE-SCALE USES OF ORGANISMS CONTAINING RECOMBINANT DNA MOLECULES.

Dr. Nightingale introduced the proposal (tabs 1096, 1101/IV, 1110, 1095/I/B) of Dr. Allan Waitz to incorporate the Physical Containment Recommendations for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules into the Guidelines as a new appendix.

Dr. Nightingale said Dr. Waitz believes such an action would provide a more efficient mechanism for further comment or change to the Recommendations. Dr. Waitz also expressed concern that failure to take such action may leave a perceived gap in the overall regulatory scheme thereby encouraging the development of conflicting regulatory requirements. He thought this action would aid the IBCs by organizing all necessary specifications in one document.

Dr. Nightingale said the Large-Scale Review Working Group, which met on October 26, 1982, forwarded this proposal to the Working Group on Revision of the Guidelines without comment.

The Working Group on Revision of the Guidelines at its January 21, 1983, meeting recommended incorporation into the Guidelines of the Physical Containment Recommendations. Dr. Nightingale pointed out that editorial modifications to revise and update sections of the Physical Containment Recommendations will be necessary if the RAC recommends this action. These minor editorial changes will reflect the modifications introduced into the Guidelines since the Recommendations were published in April 1980.

Dr. Nightingale noted that one comment on the proposed action was received (tab 1110): Dr. Irving Johnson of Eli Lilly and Company "does not see a particular need to incorporate" the Recommendations into the Guidelines.

Dr. Levine asked the industrial representatives present at the meeting to comment on the proposal. Dr. Harvey Price of the Industrial Biotechnology Association (IBA) said the IBA did not adopt a formal position regarding Dr. Waitz' proposal. Most IBA member companies support the proposal; however, some member companies disagree.

Mr. Bernard Mlynczak of Monsanto Corporation said Monsanto complies with the Guidelines and the Large-Scale Recommendations. He said Monsanto would comply whether or not the Recommendations were an appendix of the Guidelines. Dr. Pat Fallon of Hoffman-LaRoche, Inc., concurred. Dr. Wensink thought university IBCs would find it convenient to have the Recommendations as part of the Guidelines.

Dr. Nightingale moved that the Large-Scale Recommendations be included as an appendix in the Guidelines, with incorporation of the editorial modifications proposed in tab 1101/IV. Mr. Mitchell seconded the motion.

By a vote of sixteen in favor, none opposed, and one abstention, the RAC recommended the Large-Scale Recommendations be incorporated into the Guidelines as an appendix.

XV. FUTURE MEETING DATES

Dr. Gartland said the next RAC meeting was scheduled for September 19, 1983.

XVI. CLOSING REMARKS AND ADJOURNMENT

Dr. Gartland said the terms of six RAC members would terminate on June 30, 1983. The members leaving the committee are: the Chairman, Dr. Kenneth Berns; Drs. Winston Brill; Werner Maas; Elena Nightingale; James Mason; and Jean Harris. Dr. Gartland awarded certificates of service to the retiring members.

Dr. Berns said he had tremendously enjoyed working with the RAC committee and NIH staff.

The meeting was adjourned at 4:45 p.m., April 11, 1983.

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Respectively submitted,

Elizabeth A. Milewski, Ph.D.
Rapporteur

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

I hereby certify that, to the best of my knowledge, the foregoing Minutes and Attachment are accurate and complete.

Date

Kenneth I. Berns, Ph.D., M.D.
Chairman
Recombinant DNA Advisory Committee

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