

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

RECOMBINANT DNA ADVISORY COMMITTEE

MINUTES OF MEETING

APRIL 23-24, 1981

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APRIL 23-24, 1981

The Recombinant DNA Advisory Committee (RAC) was convened for its twenty-second meeting at 9:00 a.m. on April 23, 1981, in Conference Room 10, Building 31C, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20205. Mr. Ray Thornton (Chairman), President, Arkansas State University, presided. In accordance with Public Law 92-463, the meeting was open to the public from 9:00 a.m. to 4:30 p.m. on April 23, and from 8:30 a.m. to adjournment at 1:25 p.m. on April 24. The meeting was closed to the public from 4:30 p.m. to 6:00 p.m. on April 23 for the review of proposals involving proprietary information.

Committee members present for all or part of the meeting were:

Dr. David Baltimore; Dr. Kenneth Berns; Dr. Winston Brill; Dr. Allan Campbell; Mrs. Zelma Cason; Dr. Nina Fedoroff; Dr. Richard Goldstein; Dr. Susan Gottesman; Dr. Jean Harris; Dr. King Holmes; Ms. Patricia King; Dr. Sheldon Krinsky; Dr. Myron Levine; Dr. Werner Maas; Dr. James Mason; Dr. Gerard McGarrity; Dr. Robert McKinney; Dr. Elena Nightingale; Dr. Richard Novick; Dr. Ramon Pinon; Dr. John Scandalios; Dr. Luther Williams; and Dr. William J. Gartland, Jr., Executive Secretary.

A Committee roster is attached. (Attachment I)

The following ad hoc consultants to the Committee were present:

Dr. Edward A. Adelberg, Yale University; Dr. D. Michael Gill, Tufts University.

The following non-voting members and liaison representatives were present:

Dr. Charlotte Bell, U.S. Department of Justice; Dr. Chia T. Chen, OSHA, U.S. Department of Labor; Dr. George Duda, U.S. Department of Energy; Dr. Timothy J. Henry, Food and Drug Administration; Dr. Herman Lewis, National Science Foundation; Mr. Seth Pauker, National Institute for Occupational Safety and Health;

¹The RAC is advisory to the NIH, and its recommendations should not be considered as final and accepted. The Office of Recombinant DNA Activities should be consulted for NIH policy on specific issues.

Dr. Jane Shultz, Veterans Administration; Dr. Sue Tolin, U.S. Department of Agriculture; and Dr. William J. Walsh, III, U.S. Department of State.

Other National Institutes of Health staff present were:

Dr. Edward R. Applebaum, NICHD; Dr. Marilyn Bach, NIAID; Dr. Stanley Barban, NIAID; Mr. Manuel S. Barbeito, ORS; Dr. Pravin N. Bhatt, NIAID; Ms. Becky Connors, NIAID; Dr. Irving Delappe, NIAID; Dr. John Irwin, ORS; Ms. Kitty Kaplan, ORS; Dr. Richard Krause, NIAID; Dr. Elizabeth Milewski, NIAID; Dr. Stanley Nagle, NIAID; Dr. John Nutter, NIAID; Ms. Suzanne Pitts, ORS; Mr. Richard Riseberg, OGC; Dr. Bernard Talbot, OD; Dr. Michael H. Vodkin, NIAID; and Dr. Rudolf Wanner, ORS.

Others in attendance for all or part of the meeting were:

Ms. Claudia Baskin, Pharmaceutical Manufacture Association Newsletter; Ms. Tineke Bodde, BioScience Magazine; Ms. Irene Brandt, Eli Lilly & Co.; Dr. Daniel Bull, New Brunswick Science Co., Inc.; Dr. Jerry Callis, USDA; Dr. Aileen Compton, Smith-Kline & French; Mr. David Dickson, Nature; Dr. Peter Farley, Cetus Corporation; Dr. David Gelfand, Cetus Corporation; Dr. Patricia Guerry, Genex Corporation; Dr. Lowell Hamison, Office of Assistant Secretary for Health; Mr. Phil Hiltz, Washington Post; Mr. David Holzman, Freelance Writer; Dr. Paul Hung, Abbott Research Laboratories; Dr. Evelyn Hurlburt, Johns Hopkins School of Medicine; Mr. Eric Juengst, National Endowment for the Humanities; Ms. Chris Joyce, New Scientist Magazine; Dr. Attila I. Kadar, Food and Drug Administration; Mr. Alan Kaplan, Attorney, Washington, D.C.; Mr. Geoffrey Karny, Office of Technology Assessment; Mr. B. Khosrovi, Cetus Corporation; Dr. Rihito Kimura, Georgetown University; Dr. Dennis G. Kleid, Genentech, Inc.; Dr. Michael Konrad, Cetus Corporation; Dr. Walter Laird, Food and Drug Administration; Ms. Carter Leonard, Blue Sheet; Dr. Morris A. Levine, Environmental Protection Agency; Ms. Pat Lewis, Genetic Tech News; Dr. D. S. Mabry, Pfizer, Inc.; Dr. John J. Mekalanos, Harvard Medical School; Dr. James McCullough, Library of Congress; Ms. Laura Mergher, NCSM; Dr. Henry Miller, Food and Drug Administration; Dr. Bernard J. Mlynczak, Monsanto Co.; Dr. John Murphy, Harvard Medical School; Dr. Seigo Nakajira, Hamanatsu University School of Medicine; Dr. Ann Norberg, Monsanto Co.; Dr. Stephen Pijar, Food and Drug Administration; Dr. William Pilacinski, Molecular Genetics, Inc.; Dr. John Richardson, Centers for Disease Control; Ms. Sandra Ronspies, Genentech, Inc.; Dr. Michael Ross, Genentech, Inc.; Dr. B. A. Rubin, Wyeth Laboratories; Mr. Harold Schmeck, New York Times; Mr. Vincent Simmons, Genex Corporation; Mr. Dan Smith, Peoples Business Commission; Dr. Gerald Still, U.S. Department of Agriculture; Ms. Nancy Tomich, U.S. Medicine; Mr. Jeff Treuhitt, McGraw-Hill, Inc.; Mr. Charles Turbyville, Genetic Engineering Letter; and Dr. Susan Wright, University of Michigan.

I. CALL TO ORDER AND OPENING REMARKS

Mr. Ray Thornton, Chairman, called the meeting to order at 9:00 a.m., on April 23, 1981. He introduced the two ad hoc consultants who would participate in the session, Dr. Edward Adelberg of Yale University and Dr. D. Michael Gill of Tufts University.

Mr. Thornton said that the terms of several RAC members would expire with the April 1981 RAC meeting. These members are: Drs. Susan Gottesman, Allan Campbell, Luther Williams, Richard Novick, David Parkinson, Sheldon Krinsky, and Ms. Zelma Cason. Mr. Thornton extended his personal appreciation and the appreciation of the committee to the retiring members.

Mr. Thornton said a risk assessment protocol had been submitted to the NIH by Dr. Malcolm Martin of the NIH. He asked the Risk Assessment Subcommittee to evaluate the proposal. Dr. Gartland said that if the subcommittee approved the protocol, it would then be forwarded to the NIH Biosafety Committee for evaluation.

II. MINUTES OF THE JANUARY 8-9, 1981 MEETING

Ms. Cason said she had reviewed the minutes (tab 1014) of the January 8-9, 1981, meeting and found them to be correct. She moved acceptance of the minutes. Dr. Harris said she also had reviewed the minutes and found them to be substantively accurate. She seconded Ms. Cason's motion. Mr. Thornton noted that several typographical errors had been called to ORDA's attention.

Dr. Berns said that a substantive phrase had been omitted from a statement attributed to him. (January 8-9, 1981 Meeting, Item III. Meeting of the Institutional Biosafety Committee Chairpersons, page 6). He asked that this phrase be added to his statement on the proposed CDC Biosafety Guidelines for Etiological Agents (additional language in italics):

"Dr. Berns said he found the CDC's proposed biosafety guidelines for etiological agents, with regard to some of the specific containment levels suggested, to be capricious and unscientific, and the CDC unresponsive to expressed concerns."

Ms. Cason accepted, as an amendment, the proposed language. Mr. Thornton called for a voice vote, and the minutes of the January 8-9, 1981 meeting, as amended, were unanimously accepted.

III. REPORT FROM DIRECTOR, NIAID

Dr. Krause, the Director of National Institute of Allergy and Infectious Diseases (NIAID), said that the institute had recently been reorganized; as part of this reorganization, responsibility for risk assessment activities in recombinant DNA had been transferred from the Office of Specialized Research and Facilities to the Office of Recombinant DNA Activities.

Dr. Krause reported that as a follow-up to the recommendations of the NIAID Workshop on Recombinant DNA Risk Assessment held in Pasadena, California, a receipt date of July 1981 had been set for applications solicited to examine the fate of biologically active polypeptides in the human intestinal tract. In addition, NIAID had intensely advertised to identify a contractor to perform insulin autoimmunity studies. Only one proposal was received in response to the Request for Proposals (RFP); this proposal was rated unacceptable by the scientific review group.

Dr. Krause briefly commented on the U.S. - Japan meeting which was held in Hawaii in February 1981. He said the meeting was an outgrowth of a science and technology agreement between the U.S. and Japan. In the area of recombinant DNA that agreement entails sharing of information on Guidelines, risk assessment activities, etc. Four RAC members, Drs. Berns, Gottesman and Levine and Mr. Thornton, attended the Hawaii meeting. Dr. Krause said there will be a second meeting in November 1981 at the NIH dealing with the introduction of recombinant DNA into eukaryotic cells. He said the Japanese wish to host a subsequent meeting on yeast genetics. He acknowledged Mr. Justin Bloom of the U. S. State Department, currently Counselor for Scientific and Technological Affairs with the U. S. Embassy in Tokyo, as having been most helpful.

IV. PROPOSED REVISION OF GUIDELINES FOR RECOMBINANT DNA INVOLVING E. COLI K-12 AND S. CEREVISIAE HOST-VECTOR SYSTEMS

Mr. Thornton introduced Dr. Adelberg and asked him to initiate the discussion on this proposed revision of the Guidelines (tabs 998, 1015/I, 1017). Dr. Adelberg said he had originally proposed, at the November 1980 IBC Chairpersons' Meeting, that the paperwork associated with experiments covered by Section III-O be eliminated. He said he offered his proposal in light of the widely held view that experiments covered by Section III-O are "negligible in risk." In response to this request and the level of support it had received at the IBC Chairpersons' meeting and at the request of the RAC, ORDA had developed the language which appeared in the March 20, 1981 Federal Register (46 FR 17994). Dr. Adelberg said he would support any of the three published proposed options as all would reduce unnecessary paperwork. He said he personally favored option B as this option would eliminate paperwork but would require a P1 level of containment for Section III-O experiments.

Dr. Gottesman said that RAC, at the January 8-9, 1981 meeting, had indicated that diminishing the amount of paperwork associated with experiments covered by Section III-O was desirable. She said that currently, Section III-O covered experiments employing E. coli K-12 and laboratory strain Saccharomyces cerevisiae host-vector systems. She noted that RAC, at the April 1981 meeting, would review a request to include B. subtilis HVI host-vector systems under Section III-O. Dr. Gottesman briefly reviewed the three options. She favored going as far as option A, did not favor option C, and was undecided about option B.

Dr. Krinsky said the primary goals are: (1) to reduce excessive paperwork, (2) to eliminate the collection of information of no practical use, and (3) to support good laboratory practice. Dr. Krinsky said he was not certain registration of Section III-O experiments with the IBCs was useful, but he suggested such a procedure might prove useful in investigating worker illness or to uncover inadvertent misclassifications of experiments. He supported option A.

Dr. Mason said he would support option C. He suggested that RAC has acquired enough experience to justify exempting those experiments covered by Section III-O; the exemption might be tailored to meet residual concerns through the selecting of suboptions. He moved acceptance of option C. Mr. Thornton called the vote. Ten RAC members supported the motion while ten voted against it.

Mr. Thornton cast his tie-breaking vote against the motion. Dr. Scandalios indicated at this point that he had not voted, but now wanted to support the motion, making the vote eleven in favor to ten opposed.

Dr. Gottesman said, in view of the close vote on option C, it might be helpful to Dr. Fredrickson to know the sense of the RAC in regard to changing the Guidelines at least as far as option B or option A, and suggested that the RAC cast strawvotes on these options. Mr. Thornton and Dr. Berns agreed.

Dr. Fedoroff requested further discussion of the issues. She felt registration of experiments was an important aspect of the Guidelines; option A differed significantly from options B and C in entailing a registration provision.

Citing parliamentary procedure, Dr. Baltimore said a motion should be perfected through amendments and selection of suboptions before any vote is called. Mr. Thornton agreed with Dr. Baltimore but indicated that in this case he had hoped to expedite discussion by gauging the sentiment of the RAC in straw votes; RAC would subsequently amend and perfect the language.

Ms. King said that if other RAC members wished to call attention to procedural problems, she felt obliged to indicate that the previous vote on Dr. Mason's motion contained two procedural irregularities: (1) the chair had not called for discussion before the vote, and (2) a vote (Dr. Scandalios') was noted after the RAC vote had been tallied.

Mr. Thornton made the following ruling: The chair ruled that the votes on Dr. Mason's motion were cast ten in favor, ten opposed. The chair broke the tie by casting his vote against the motion. The vote noted after the tally was not accepted. Consequently, the motion, as made, failed. Mr. Thornton said he would entertain appeals on this ruling of the chair. As no appeal was made, he said the RAC should now proceed following strict parliamentary procedure, rather than taking straw votes.

Dr. Novick moved adoption of option B with the proviso that a listing requirement be appended, but that no review be required. Dr. Fedoroff seconded the motion. Ms. King pointed out that supporters of option C could move a substitute motion at this point. She said she herself would not do so, as she would not support option C. Dr. Brill moved substitution of option C. The motion was seconded. Mr. Thornton said that the substitute motion would be considered before the original motion, but that it should be perfected before a vote to adopt would occur. In order to expedite discussion, Mr. Thornton asked Dr. Brill, as the maker of the substitute motion, to select the suboptions he would prefer under option C. Dr. Brill said he would prefer suboptions C-1-a, C-2-b, C-3-b, C-4-c, and C-5-b.

Dr. Gottesman suggested that suboption C-1-b be substituted for suboption C-1-a. She said suboption C-1-a is much broader than Section III-O of the current Guidelines; it would permit the use of all vectors including conjugation proficient plasmids and vectors. Dr. Brill accepted Dr. Gottesman's amendment.

Dr. Berns suggested that suboption C-2-a, in which large-scale experiments are not exempt, be substituted for suboption C-2-b, which would exempt large-scale experiments. Dr. Brill accepted the substitution.

Dr. Berns also suggested that suboption C-3-a be substituted for C-3-b, as he thought good microbiological procedures should be recommended. Dr. Brill accepted this substitution.

Dr. Berns further suggested an amendment to option C; cloning of CDC Class 3 agents in host-vector systems covered by Section III-O, would be exempt provided that not more than 75 percent of the genome is cloned. Dr. Baltimore asked if this provision would apply to bacteria as well as to viruses. Dr. Berns said he had made the proposal with viruses in mind. Dr. Fedoroff said the amendment did not clearly indicate whether less than 75 percent of the genome could be used to construct a library or if less than 75 percent of the genome was permissible in a single clone. Dr. Berns said he visualized his proposal as applying to a single clone or a single cell. There was no second to Dr. Berns' amendment.

Dr. Gottesman moved to substitute suboption C-4-b for suboption C-4-c. Dr. McKinney asked Dr. Adelberg how suboption C-4-b would impact on his institution. Dr. Adelberg replied that in his IBC experience, experiments involving CDC Class 3 agents constitute a very small fraction of all recombinant DNA work. Dr. McGarrity noted that suboption C-4-b requires review by either the IBC or an institutional official. He asked that the reviewing official be stipulated. Dr. Gottesman agreed and specified review by the IBC. By a vote of eleven in favor, four opposed, and five abstentions, RAC adopted suboption C-4-b.

Dr. Campbell offered an amendment to suboption C-4-b. The following sentence would be added:

"If there is any chance that the original Class 3 agent can be regenerated from the cloned DNA, the containment level shall be no lower than that appropriate for the agent itself."

Dr. Gottesman seconded the amendment. By a vote of twenty in favor, none opposed, and one abstention the amendment was adopted.

Suboption C-5-b, eliminating prior review requirements for experiments involving deliberate expression of eukaryotic genes, was discussed by the RAC. No proposal was advanced to substitute suboption C-5-a for suboption C-5-b.

Mr. Thornton said the next vote would occur on the perfected substitute motion, i.e., option C with suboptions C-1-b, C-2-a, C-3-a, C-4-b with the added Campbell sentence, and C-5-b. By a vote of thirteen in favor, eight opposed, and no abstentions the RAC substituted this perfected motion.

Mr. Thornton then called the vote on the motion. By a vote of thirteen in favor, eight opposed, and no abstentions, the RAC adopted the motion.

V. PROPOSED BIOSAFETY GUIDELINES FOR MICROBIOLOGICAL AND BIOMEDICAL LABORATORIES

Mr. Thornton introduced Dr. John Richardson of the Centers for Disease Control to brief the RAC on the status of the "Proposed Biosafety Guidelines for Microbiological and Biomedical Laboratories." Dr. Richardson said that a notice soliciting public comment had been issued in the Federal Register in October 1980. Since that announcement, approximately 300 written comments were received by the NIH and the CDC.

Most responses from industry and state health departments were supportive of the underlying philosophy, although some reservations were expressed concerning classifications of particular agents, or of recommended practices at a particular containment level. Responses from academic institutions and many clinical laboratories did not support either the philosophy of a voluntary national guideline or the classifications of many of the etiological agents or some of the work practices and facility recommendations. Many of the academic institutions and some of the clinical laboratories see the proposed guidelines as the first step in the development of federal regulations. Many academic institutions are concerned about possible premature implementations of the proposed guidelines by IBCs. Also, concern has been expressed that the time provided for public comment is insufficient.

In response to these concerns, Dr. Richardson said that public comments received after April 15 will also be considered. He said that there is no existing Public Health Service authority under which intrastate laboratory activities could be regulated, and that neither NIH or CDC is interested in assuming this task.

Dr. Richardson said that at least one revision, incorporating comments, and very likely two or more additional revised drafts will be prepared, and will be subjected to a reasonable review process. He felt that the guidelines were philosophically appropriate for the NIH and the CDC; the guidelines are only guidelines, they are voluntary and hopefully they will result in an upgrading of laboratory functions.

Dr. Richardson said the proposed guidelines were initiated with two purposes in mind:

- (1) to modify the current Classification of Etiological Agents on the Basis of Hazard, and
- (2) to use the guidelines as an "in-house" guidance document for CDC staff in the absence of any other document of similar nature. He pointed out that had comment on the guidelines not been solicited, the "in-house" CDC document would have become the national standard by default.

Dr. Brill said he supported the concept of guidelines for the handling of potentially dangerous organisms. He said, however, that the guidelines will become "regulations" as many institutions would require investigators to abide by them. Dr. Baltimore said that questions of legal liability ensure that the guidelines will be in a sense "regulations;" they would legally be regarded as standard practice. For this reason every recommendation in the guidelines should be carefully considered.

Dr. Levine noted that the guidelines specify containment levels for "activities involving the use or manipulation of large quantities or high concentrations of cultures or other materials known or suspected of containing the agent." He asked if "large quantities" or "high concentrations" had been defined, and if not, who would determine this. Dr. Richardson replied that the guidelines in this respect are meant to be interpretive and judgemental. CDC would never propose to set any stated quantity as representing a "large quantity" for all microorganisms. Much concern has been expressed over this aspect of the guidelines however, and attempts may be made in the revision to be more descriptive of criteria to be used in determining what constitutes a "large quantity" or "high concentration" of an agent.

VI. PROPOSAL TO CONVERT NIH GUIDELINES INTO A CODE OF STANDARD PRACTICE AND TO REDUCE RECOMMENDED CONTAINMENT LEVELS FOR SOME EXPERIMENTS

Mr. Thornton said he would recognize Dr. Nightingale who had requested an opportunity to present her views on the proposal (tabs 994, 1015/II, 1017) offered by Drs. Baltimore and Campbell.

Dr. Nightingale said it had been apparent for some time that the committee perceived a need for a reassessment of the purpose of the Guidelines. The proposal from Drs. Baltimore and Campbell is a serious attempt to meet

that need, and since the proposal had been published in the Federal Register, action might be taken on it today. She said she herself doubted the wisdom of this approach. She suggested rather that a subcommittee of RAC, selected with a balance of expertise and perspective, should study the issues and present to the RAC at a future meeting an array of policy options on the existence, nature, and content of the Guidelines. Such a procedure is more likely to lead to decisions that are balanced, well-thought through and have maximum potential to be perceived as such by those not engaged in the research in question.

Dr. Campbell said he thought his proposal was an appropriate action at this time. Removing the regulatory aspects of the Guidelines is coupled to reducing the required containment levels. He stated that he would not support eliminating the regulatory aspects of the Guidelines if he believed there was some hazard worth regulating.

Dr. Campbell then discussed various aspects of the proposal. He said the proposal did not specify any containment level on the basis of the cloned segment's origin; that omission is deliberate. He and Dr. Baltimore felt that, except for the few cases covered under the prohibitions, a small segment of DNA inserted into a nonpathogenic host-vector system would not create a pathogen. Appropriate levels of containment for a pathogenic host-vector system would, on the other hand, be specified by the CDC Guidelines.

Dr. Campbell then called the committee's attention to a sentence in Part III of the proposal:

"As a general practice, investigators should use the highest level of biological containment (HV3 > HV2 > HV1) which is available and appropriate for the purposes of the experiment."

He said he viewed the sentence as an admonition for simple prudence; where more biologically contained systems are available, and their use doesn't interfere in any way with performing the experiment, their use should be encouraged. On the other hand some feel this sentence is too strong; they say where there is no perceived hazard it is silly to be telling people to use higher containment levels.

Dr. Campbell said the proposal would eliminate most of Part IV which specifies procedures, but retain Section IV-E which defines RAC composition and procedures. Part VI of the Guidelines dealing with voluntary compliance would be eliminated, as all of the Guidelines would be voluntary, except for the sections dealing with protection of proprietary information voluntarily submitted. The prohibitions specified in the current Guidelines would still apply. He said he and Dr. Baltimore felt each prohibition should be discussed on an individual basis.

Dr. Baltimore added that he saw the maintenance of the RAC as very important. The most important RAC function would be to maintain surveillance

over recombinant DNA. RAC would also continue to deal with issues surrounding the prohibitions. He hoped RAC could deal with general issues of bio-hazards and would aid CDC in formulating the CDC guidelines. Dr. Baltimore said the proposal would not greatly affect industry as industry has voluntarily accepted adherence to the Guidelines. Lastly, Dr. Baltimore quoted from a letter from Dr. Zinder (tab 1017). "It would be an important precedent for the NIH to dismantle the unneeded regulatory structure. If scientists are ever again to attempt to cope with potential hazard, they must see that what they believed were temporary measures can be undone."

Dr. Harris said that examination of the RAC's actions in the last two years indicates a general movement in the direction the authors propose. Although she agreed in principle with the rationale supporting the proposal, she said she felt waiving NIH and RAC responsibilities in this area is a giant leap deserving very careful evaluation and consideration. She agreed that little evidence to date supports the general public concern about potential hazards of recombinant DNA, but pointed out that public concern and distrust of bioprocessing technologies continues, particularly with those technologies which lend themselves to human genetic applications. The RAC and the NIH Guidelines both have served to inspire public trust and confidence. She said it is her strong conviction that the Guidelines have precluded precipitous regulation by local governing groups, by other federal agencies, and by Congress; regulations which, if enacted, would have stifled innovation. Premature abrogation of the Guidelines might skew future regulation and public policy.

Dr. Harris said she personally is more comfortable with the recommendation to reduce containment levels than with the proposal to convert the Guidelines to a code of standard practice. She proposed a two-stage process in which RAC would consider the recommended reduction in containment levels, while postponing consideration of converting the Guidelines into a non-regulatory code. Dr. Fedoroff and Ms. King endorsed Dr. Harris' statement.

Ms. King noted Dr. Zinder's plea that scientists "must see that what they believed were temporary measures can be undone," but also added that the public must see that if the RAC undoes the Guidelines, it does so in a responsible manner. She agreed that the Guidelines should be reassessed and reevaluated. She suggested that RAC or a RAC subcommittee should undertake such a reevaluation. She urged that public input should be solicited, and observed that a Federal Register announcement is not adequate. She felt debate with input from other sectors is important in formulating a recommendation concerning the Guidelines.

Dr. Nightingale said the process by which the status of the Guidelines would be altered will greatly influence public acceptance of that alteration. Dr. Harris said that process is of primary importance because if the public perceives the action as inappropriate, a backlash could result. She said the process will entail education of concerned citizens. Dr. Brill and Ms. King expressed the belief that there is very little public distrust of the scientific community in the area of recombinant DNA. The public is

awaiting the benefits of the technology. Dr. Nightingale said the impression one receives depends on whom one talks to: she pointed to recent congressional hearings on the misbehaviour of certain scientists as evidence of a growing distrust of the scientist.

Dr. Campbell said the RAC must decide whether its function is to deal with danger or to deal with fear. He believed RAC's function is to deal with danger. He said that in his judgement, by maintaining the Guidelines as presently constituted, RAC was delivering to the public the message that a group of responsible, serious, informed people perceive a danger which should be regulated.

Ms. King said that the question of reducing containment should not be uncoupled from the issue of converting the Guidelines to a voluntary code of standard practice. She said the path which RAC has been following, one of piecemeal erosion, would eventually result in an empty facade. At some point along that path, however, deliberate discussion and consideration of the process should be undertaken. In such a discussion, the issue of reducing containment is intimately linked to the conversion of the Guidelines to a voluntary code. Dr. McKinney supported a deliberate reassessment of the Guidelines.

Dr. Gottesman said she saw the Baltimore-Campbell proposal as having three parts: (1) Elimination of the penalties from the Guidelines, which need not be coupled with other changes, would move academia into the industrial mode. (2) Other procedural changes recommended, i.e., eliminating IBCs, eliminating registration, etc. (3) Lowering of containment conditions. She said she personally would prefer to simplify some of the procedures without necessarily lowering all containment requirements to P1.

Dr. Novick summarized his opinions as follows: (1) He strongly supported the notion of prudence in biological research. (2) He felt the notion of guidelines in this area is entirely correct and appropriate. (3) At this stage in their evolution, a review and reassessment of the Guidelines was appropriate. (4) He very much wanted to see a uniform standard applied to both industry and academia.

Dr. Baltimore reiterated his views on coupling of lowering of containment to conversion of the Guidelines to a code of standard practice. If the RAC agrees on lowering of containment to P1, then the complicated regulatory edifice is unnecessary.

Dr. Holmes said that groups either exert internal control or they invite societal regulation from the greater community. He suggested that RAC learn the sentiment of the scientific community; if a substantial minority of scientists opposes deregulation and deregulation occurs, the scientific community invites societal control from without.

Dr. Susan Wright was recognized by Mr. Thornton. Dr. Wright offered the following observations. She said she has no particular axe to grind about recombinant DNA techniques, but she does have a historical perspective to

which she is committed. She said there have been two serious violations of the Guidelines in the past year, and she thinks it is a reflection of the lack of concern of this committee that those items are not on the agenda before proposals to relax the Guidelines. She felt that dismantling the mechanisms that have been set up to enforce the Guidelines will signal the very small minority of scientists who pursue their research goals irresponsibly that high standards in research are no longer a concern. In addition, she felt that many scientists, perhaps the majority, would maintain that deliberate construction of hazardous organisms by recombinant DNA techniques is possible and is of concern. In view of these considerations, she felt the present system of controls, specifically the IBCs and their links to NIH, is important for the following reasons: (1) The IBCs constitute an important screening device for detecting hazards, and ensuring that experiments which entail hazards are performed under suitable conditions. (2) The IBCs serve as an important reminder to researchers that their peers and community members take the safety of their work seriously. (3) The IBCs can serve as bodies to which employees can turn if they believe that research is being improperly conducted. This last point is very important to workers who are not represented by trade unions.

Dr. Wright contended that the cost in money and time of registration of these experiments is insignificant compared to all the other paperwork that U.S. researchers are asked to undertake. The benefits in the maintenance of high standards and the avoidance of harmful experiments are very substantial and should be taken seriously in any society that is committed to the protection of the interests of all its members, not only the interests of those having the greatest access to the decision-making arenas. The history of regulation of other fields, for example, drugs, auto safety, health and safety in the workplace, shows that, in general, controls were not introduced until the social costs of accidents had become very high, really intolerable. Each potentially avoidable injury or death represents a cost in human suffering which cannot be absorbed into any cost-benefit equation. One of the original motives for the controls for recombinant DNA technology was to avoid repeating that pattern in this new field. She hoped the RAC members would maintain that commitment as they proceed to make a decision on this proposal.

Dr. Baltimore said that P1 is a much higher standard of laboratory conduct than has historically been common practice. Most RAC members appear to accept the notion that there is minimal perceptible hazard associated with recombinant DNA. The RAC should attempt to construct Guidelines reflecting that view.

Dr. Krinsky said that our society is facing an explosion of biological technology which will result in the exposure of more individuals to more types of organisms in more facilities. He felt there has been a paucity of controlled experiments to test the hypothesis that there is no risk beyond that associated with other types of biological research. That conclusion has been drawn on the available information, but not from a systematic set of experiments.

Dr. Mason said that accountability and responsibility, in the ethical, moral, and legal sense, rest ultimately with the institution and the principal investigator. Regulatory guidelines will not stop the person who is dishonest or malicious or careless. He agreed with the observation that a "piecemeal" erosion of the Guidelines was occurring. He suggested that piecemeal erosion if it continues too long without reexamination of the basic premises, can result in a loss of respect for the institution promulgating an eventually empty statement. He endorsed the Baltimore-Campbell proposal and moved that a RAC subcommittee be established with the specific mandate of evaluating that proposal and reporting back to the RAC on the implementation process. Dr. Berns seconded the motion.

Dr. Gottesman preferred that the RAC subcommittee not be tied to the Baltimore-Campbell proposal per se but that it consider many options in a reevaluation of the need for changes in procedures, penalties, and containment levels of the Guidelines. Dr. Novick agreed with Dr. Gottesman. He moved that a subcommittee be instituted to review the Guidelines with respect to both general containment levels and the proposal to convert the Guidelines to a voluntary code. Dr. Gottesman seconded the motion.

Dr. Williams suggested Dr. Novick's motion be amended to direct the subcommittee to examine the Baltimore-Campbell proposal during its deliberations.

Dr. Harris, with Dr. Nightingale, offered the following substitute motion:

"That RAC recommend to the Director, NIH, that a study group comprised of RAC members, and any others he so directs or appoints, be constituted to review the current regulations. Such review to include but not be limited to: (a) the present need for the Guidelines in their existing form and procedures, as opposed to a voluntary standard of practice, (b) the continued applicability of the present Guidelines to recombinant DNA technology, (c) the currently recommended levels of containment, (d) current processes and procedures impeding or facilitating research and/or industrial application, and (e) mechanisms for soliciting public input. Such study group to report to the RAC and the Director, NIH, its finding, conclusions and recommendations for RAC review and recommendation."

Dr. Nightingale said that the mechanisms for soliciting public comment were to include processes beyond publication in the Federal Register.

Dr. Goldstein asked if Dr. Harris' proposal would relieve RAC of responsibility for the reevaluation of the Guidelines. Dr. Harris replied that the initiative would remain with the RAC, but that the NIH Director might wish to expand the review group to include non-RAC members. The working group would report to RAC, which would then offer recommendations to the Director. Dr. Goldstein suggested the language be amended to indicate clearly that the working group is a RAC subcommittee. Dr. Harris agreed and substituted the following sentence:

"The Chairman of the RAC appoint a study group comprised of RAC members and others deemed appropriate."

Dr. Campbell asked whether the working group would actually solicit public comment or whether it would offer suggestions to RAC on mechanisms for soliciting comment. Dr. Harris envisaged the working group offering suggestions on mechanisms. Dr. Campbell said he would prefer the working group begin soliciting comment and suggested the motion be rephrased to state this. Dr. Harris agreed. Dr. Williams asked if comment would be solicited before recommendations are formulated. He felt it was more practical to seek input after a recommendation has been formulated.

Dr. Harris replied that she originally envisaged the working group formulating recommendations, and RAC subsequently soliciting comment. She was not, however, adverse to Dr. Campbell's proposition. Dr. McKinney suggested that RAC should not hamstring the working group with specific guidance as to how to obtain public input.

Mr. Pauker suggested that evidence supporting the premise that there is no risk in recombinant DNA manipulations beyond those due to the constituent parts, should be assembled and critically evaluated. It should also be publicly available. Dr. Goldstein supported this position and asked if Dr. Harris would add this to her motion. Dr. Harris replied that her proposal already implicitly included this change.

Dr. Williams asked if Dr. Harris would accept an amendment deleting the reference to soliciting public input. She replied she would not. Dr. Gottesman offered an amendment which would delete item (e) from its current place in Dr. Harris' proposal and instead add at the end of the proposal the sentence: "RAC will solicit public comment on this proposal."

Dr. Norberg of Monsanto Corporation pointed out that the NIH Guidelines were not regulations and questioned whether language identifying them as regulations should be included in Dr. Harris' proposal. Dr. Harris replied that she meant the Guidelines as currently constituted versus a standard code of practice, and amended the language to eliminate any reference to "regulations."

Dr. Campbell requested a clarification of the amended language proposed by Dr. Gottesman. He suggested that the last sentence should read: "Public comment will be solicited on the proposal." Drs. Gottesman and Harris agreed.

Mr. Thornton called the vote on Dr. Harris' amended substitute motion. Dr. Harris reread the motion as follows:

"The Chairman of the RAC appoint a study group composed of RAC members and any others deemed appropriate to review existing recombinant DNA Guidelines. Such review to include but not be limited to: (a) the present need for the Guidelines in their existing form and procedures, as opposed to a voluntary standard

of practice; (b) continued applicability to recombinant DNA technology; (c) currently recommended levels of containment; (d) current processes and procedures impeding or facilitating research and/or industrial application. Such study group to report back to the RAC its findings, conclusions, and recommendations for RAC review and consideration. Solicitation of public input and comment beyond publication in the Federal Register will be obtained."

By a vote of fifteen in favor, four opposed, and two abstentions, the committee accepted this substitute motion. Mr. Thornton then called the vote on the motion. By a vote of nineteen in favor, two opposed, the RAC adopted the motion.

VII. PROPOSED CONTAINMENT CONDITIONS FOR FLIES

Dr. McGarrity introduced the proposal (tab 1004) from Dr. Thomas Maniatis of Harvard University to transform Drosophila with the Drosophila alcohol dehydrogenase gene. The cloned DNA (in lambda, plasmid or cosmid vectors) will be injected into either the abdomen of adult female alcohol dehydrogenase deficient flies, or into early stage alcohol dehydrogenase deficient embryos. The Guidelines allow such work to proceed at P1 containment. The Harvard IBC believes the safety measures proposed meet the requirements of P1, but asked for RAC concurrence. The containment conditions include: (1) Bottles containing flies will be disposed of by autoclaving. (2) Flies will be propagated in bottles or vials. (3) Only experienced personnel will handle the Drosophila. (4) All manipulations of adult flies will be performed in a cold room. The cold environment will act as an anesthetic for the flies. (5) All propagation of transformed flies will be carried out in an approved P1 laboratory.

Dr. McGarrity said the precautions to be employed appear to be adequate and recommended approval. Dr. McKinney said that installation of an air curtain on the entry to the cold room would provide an additional barrier against escape. Dr. Gottesman felt the precautions specified in the proposal were adequate and moved acceptance of the proposal. Dr. Fedoroff seconded. By a vote of nineteen in favor, none opposed, and no abstentions, the RAC recommended adoption of the proposal.

VIII. CONTAINMENT LEVELS FOR RECOMBINANT DNA EXPERIMENTS INVOLVING BACILLUS SUBTILIS

Dr. Williams initiated review of the request (tabs 1005, 1007, 1011, 1015/VI, 1016) submitted by Dr. Donald Dean of Ohio State University. Dr. Dean requested consideration of the current classification of Bacillus subtilis host-vector systems. Dr. Dean's request consisted of three parts:

- (1) that any asporogenous Bacillus subtilis strain which does not revert to a sporeformer with a frequency greater than 10^{-7} can

be used for cloning DNA from any nonprohibited sources, using vectors indigenous to B. subtilis, under the same conditions specified by RAC for E. coli K-12 and S. cerevisiae host-vector systems.

- (2) Bacillus subtilis strains that do not carry an asporogenous mutation can be used with vectors indigenous to B. subtilis for the cloning of DNA from any CDC Class 1 organism under P2 conditions.
- (3) Bacillus subtilis strains that do not carry an asporogenic mutation can be used with vectors indigenous to B. subtilis under P1 conditions for the cloning of DNA from any Class 1 Bacillus species.

Dr. Williams said that Bacillus subtilis is probably the most extensively understood gram-positive organism, both genetically and biochemically. It is capable of both generalized and specialized transduction and has been widely used in the industrial sector in the production of an array of antibiotics. It may be particularly well suited for certain types of recombinant DNA experiments, as Bacillus strains have the capacity to secrete a variety of proteins. Bacillus subtilis is nonpathogenic and is not known to exchange genetic information with pathogens.

Dr. Williams directed the committee's attention to data comparing the survivability of B. subtilis and E. coli K-12 in soil or water samples; E. coli survives better than B. subtilis over a five day period. Additional data demonstrate that B. subtilis spores placed in a mammalian intestine rarely sporulate, and if they do the vegetative cells quickly die.

Dr. Williams recommended approval of the request. Dr. Holmes seconded the motion.

Dr. Gottesman, noting that currently certified HVI B. subtilis host-vector systems only employ certain specified plasmids, pointed out that Dr. Dean's proposal would also permit use of phage vectors. She requested additional information concerning the proposed phage vectors. Dr. Dean replied that the host ranges of the Bacillus phages are very narrow. In his experience, transformation affords greater possibilities and avenues of genetic exchange.

Dr. Goldstein asked if B. subtilis engineered to excrete recombinant proteins is a concern. Dr. Novick replied that B. subtilis does not colonize the mammalian gastrointestinal tract. He personally did not regard the excretion of cloned proteins into soil as potentially hazardous.

Dr. Talbot requested clarification of Dr. Williams' motion. He asked if the intent was that the asporogenic B. subtilis strains would be exempted from the Guidelines as had been recommended by the RAC for E. coli K-12 and S. cerevisiae earlier in the meeting. Dr. Williams replied that he intended they would. Noting that the language of the proposal would permit the use of any "indigenous vector," Dr. Talbot questioned whether the

motion is limited to those vectors listed by Dr. Dean. Dr. Williams replied that it would not be limited. Dr. Talbot said that current certification specifications for B. subtilis HVI systems require that data demonstrating a reversion frequency to sporogony of less than 10^{-7} , be evaluated by NIH; he questioned whether this specification was implied under Dr. Williams' motion. Dr. Williams said it was not.

Dr. Gottesman asked if in permitting use of all indigenous vectors, RAC might inadvertently authorize the use of an extremely broad host range vector which might infect Bacillus pathogens.

Dr. Dean said that his proposal could be modified to eliminate the use of phage vectors that infect CDC Class 2 Bacilli such as Bacillus anthracis. It was pointed out that Bacillus cereus also is a pathogen. Dr. Levine said that Bacillus cereus enterotoxin causes disease, particularly in Southeast Asia, where it is a major cause of enteric problems. The organism produces and excretes a potent enterotoxin which contaminates foodstuffs.

Dr. Gottesman suggested the phrase "indigenous plasmid and phage vectors, whose host-range does not include Bacillus cereus and Bacillus anthracis" be substituted for the words "vectors indigenous to B. subtilis." Dr. Williams accepted this modification of the motion.

Mr. Thornton called for the vote on the amended motion. By a vote of twelve in favor, none opposed, and five abstentions, the RAC recommended the motion.

IX. CERTIFICATE OF APPRECIATION TO MRS. BETTY BUTLER

Mr. Thornton announced that Mrs. Betty Butler, who has worked for ORDA for many years, had recently accepted another position at NIH. Mr. Thornton, calling the attention of the committee to Mrs. Butler's many years of service to the RAC, which have contributed so much to its efficient functioning, presented to her a plaque in appreciation of her services, signed by NIH Director Donald S. Fredrickson.

X. EXPRESSION OF FOOT AND MOUTH DISEASE VIRUS PROTEINS IN SACCHAROMYCES CEREVISIAE, BACILLUS SUBTILIS, AND MAMMALIAN TISSUE CULTURE

Dr. Berns said he preferred to divide the Genentech, Inc., proposal (tabs 999, 1015/VII) into four parts, each to be discussed separately.

Dr. Berns first discussed the question of removing additional clones of the Foot and Mouth Disease Virus (FMDV) genome from the Plum Island Animal Disease Center. Previous NIH approval of the project permitting clones contained in E. coli K-12 to be removed from Plum Island, stipulated that the plasmids removed from Plum Island should not separately or collectively represent more than 75% of the FMDV genome. Unfortunately, the plasmids

transferred from Plum Island to the Genentech, Inc., facilities in California apparently did not contain the VP3 coding region, the VP3 protein being the predominant antigenic moiety for the virus.

The VP3 region of several FMDV serological types were subsequently cloned on Plum Island. Genentech, Inc., now requests permission to remove these additional clones to their facilities in South San Francisco. If these clones are removed, however, more than 75% of the FMDV genome will have been shipped from Plum Island. Discussion between the RAC working group on FMDV, USDA, and Genentech, Inc., led to the proposal that plasmids representing sequences to the right of base pair 6000 be returned to Plum Island, after which it would be permissible to ship from Plum Island to Genentech the plasmids of interest.

It was pointed out that the RAC recommendation at the last meeting, accepted by the NIH Director, allows the working group to approve the removal of these clones from Plum Island without obtaining full RAC concurrence. Nevertheless, Dr. Berns moved that Genentech, Inc., be granted permission to return those clones representing the extreme right portion of the genome to Plum Island, and in exchange be permitted to remove the requested clones containing the center of the FMDV genome. Dr. McGarrity seconded the motion.

The motion was adopted by a vote of sixteen in favor, none opposed, and two abstentions.

Dr. Berns proceeded to that portion of the request dealing with use of host-vector systems other than E. coli K-12. Dr. Berns said Genentech, Inc., had requested permission to clone the FMDV genome in B. subtilis host-vector systems. A discussion between the RAC working group on FMDV and representatives of Genentech, Inc., led to agreement that this would be limited to those portions of the FMDV genome lying between base pairs 500 and 4,100. He suggested that P1 conditions are adequate and so moved. Dr. McGarrity seconded the motion. By a vote of sixteen in favor, none opposed, and two abstentions, the motion was adopted.

Dr. Berns said the same type of experiment was proposed utilizing Saccharomyces cerevisiae host-vector systems. He again suggested that the experiments be permitted under P1 physical containment conditions if the subgenomic FMDV segments were restricted to those sequences which map between 500 and 4,100 of the FMDV genome. Dr. McGarrity seconded the motion. By a vote of sixteen in favor, none opposed, and two abstentions, the RAC adopted the proposal.

Dr. Berns then turned the discussion to the proposal to clone portions of the FMDV genome, using the SV40 genome as a vector, in mammalian cell culture. Dr. Berns noted that tissue culture systems are suitable "hosts" for large numbers of different types of picornaviruses. He questioned whether an adventitious recombination between a contaminating picornavirus and the hybrid SV40 - FMDV molecule might occur. Dr. Baltimore said evidence demonstrating recombination among homologous picornaviruses is marginal. He did not know of experiments in the literature looking for

recombination among heterologous picornaviruses. He added that he knew of no human virus similar by nucleic acid homology to FMDV, but some rhinoviruses appear to be similar structurally to FMDV.

Dr. Goldstein asked that FMDV disease be described. Dr. Callis of the Plum Island Animal Disease Center said that the virus does not normally cause a high mortality in adult animals. FMDV causes death among young animals, but in adults causes weight loss and, as it infects secretory cells in the mammary glands, disrupts milk production. The disease spreads very, very rapidly and can infect every barnyard animal except the horse.

Dr. Callis said that FMDV can chronically infect tissue culture systems and that many types of cells in culture, including primate cells, may be infected with FMDV.

Dr. Berns said Genentech, Inc., had requested permission to perform this type of experiment at their facilities in South San Francisco. Noting that the laboratories at Plum Island were already working with whole FMDV, he moved to permit these experiments at Plum Island under P3 containment conditions. The FMDV genome between base pairs 500 and 4,100 may be cloned subject to the RAC Working Group evaluating individual experiments prior to their initiation.

Dr. Ross asked if RAC could indicate what sort of data it would require before it would allow transfer of the material from Plum Island to California for the production phase, should the tissue culture system be successful. Dr. Gottesman said that if the motion were adopted, and the work on Plum Island were successful, RAC would want to review these results before approving transfer of material to California.

Mr. Thornton called the vote on Dr. Berns' motion. By a vote of seventeen in favor, none opposed, and one abstention, RAC adopted the motion as follows:

"Permission is granted in principle to propagate in mammalian cell culture recombinant DNA molecules consisting of segments of Foot and Mouth Disease Virus and SV40 deletion vectors under P3 conditions at the Plum Island Animal Disease Center. Approval of individual experiments is subject to review by a RAC Working Group."

XI. CLOSED SESSION

The RAC went into closed session to consider proposals from commercial concerns for scale-up of recombinant DNA experiments.

XII. CONTAINMENT LEVELS FOR RECOMBINANT DNA EXPERIMENTS INVOLVING NEUROSPORA CRASSA

Dr. Brill introduced the request (tabs 995, 1015/III) from Dr. David Perkins of Stanford University. Dr. Perkins proposed that the following language be substituted for entry 2 of Appendix E:

"Unmodified laboratory strains of Neurospora crassa can be used in all experiments for which HVI N. crassa systems are approved, provided that only DNA from Class 1 agents is used. For agents other than Class 1, unmodified laboratory strains of N. crassa can be used in all experiments for which HVI N. crassa systems are approved, providing that these are carried out at physical containment one level higher than required for HVI. However, if P3 containment is specified for HVI N. crassa, this level is considered adequate for unmodified N. crassa. Care must be exercised to prevent aerial dispersion of macroconidia in accordance with good laboratory practice.

"Mutationally modified strains of N. crassa specified as HVI in Appendix D can be used in all experiments for which HV2 N. crassa systems are approved, provided that only DNA from Class 1 agents is used."

Dr. Brill said that N. crassa is not known to be a pathogen. It is not closely associated with man or other organisms in nature. It produces no known toxins. He moved acceptance of the request. By a vote of ten in favor, none opposed, and five abstentions, the RAC adopted the motion.

XIII. REQUEST TO EMPLOY A CONJUGATIVE PLASMID TO TRANSFER NEUROSPORA CRASSA DNA

Dr. Gottesman introduced the request (tabs 1006, 1015/XIII) of Dr. Norman Giles of the University of Georgia. Dr. Giles requested permission to use a conjugative plasmid to transfer the Neurospora crassa qa-2 gene among E. coli K-12 strains. The qa-2 gene would be ligated into a derivative of the mobilizable plasmid RSF2124. The work would be performed under P2 containment conditions.

Dr. Gottesman said that although this request involved the use of a conjugative plasmid, she would support the proposal as the N. crassa qa-2 gene is a relatively well-defined DNA fragment.

Dr. Gottesman moved approval of the proposal. By a vote of seventeen in favor, none opposed, and no abstentions, RAC adopted the motion.

XIV. REQUEST TO USE AN E. COLI STRAIN CONTAINING A Mu PHAGE INSERTION

Dr. Goldstein introduced the request (tabs 1009, 1015/XII) of Dr. Darold Holten of the University of California at Riverside. Dr. Holten requested permission to utilize the E. coli K-10 strain DF214 (or derivatives thereof), and EK plasmid vectors (e.g., pBR322, pBR325) to clone rat cDNA. Strain

DF214, a K-12 derivative, contains Mu phage insertions in the phosphoglucose isomerase gene and in an unidentified location. Dr. Goldstein said the probability of the Mu lysogen transducing out the rat DNA is very low and suggested the experiment be permitted. Dr. Gottesman agreed and suggested that the initial shotgun experiments screening the rat cDNA library be conducted at P2 physical containment. After the clone of interest has been selected, work may proceed at the P1 level of containment. Dr. Goldstein moved acceptance of the proposal with Dr. Gottesman's stipulation. Dr. Novick seconded the motion.

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

XV. REQUEST FOR APPROVAL OF GUIDELINE CHANGES INVOLVING STREPTOMYCES

Dr. Levine introduced the proposal (tabs 1012, 1015/IV) submitted by Dr. Stanley Cohen of Stanford University. Dr. Cohen requested that Streptomyces coelicolor and the related organisms with which S. coelicolor naturally exchanges genetic information (S. lividans, S. parvulus, and S. griseus) be approved as HVI hosts. Streptomyces plasmids SCP2, SLPL.2, pJ101, actinophage phi C31, and their derivatives would be used as vectors.

Dr. Levine said the Streptomyces are nonpathogenic; no known hazard has been associated with large-scale industrial use of the organisms. Dr. Levine moved approval of the proposal. Dr. Fedoroff seconded.

Dr. Gottesman said the proposal was vague; the strains proposed for certification are not as well defined as some of the HVI systems approved in the past. Little data concerning known exchange mechanisms with other organisms have been included in the proposal. Furthermore, the actinophages, which are requested to be allowed as vectors, apparently have broad host ranges.

As no further comment was made, Mr. Thornton called the vote. By a vote of ten in favor, none opposed, and eight abstentions, the RAC adopted the motion.

Dr. Gottesman introduced the second proposal (tabs 1012, 1015/V) from Dr. Cohen. Dr. Cohen requested that the following entry be added to Appendix E:

"Experiments involving the cloning of DNA among members of the genus Streptomyces are permitted under P1 conditions. For these experiments, no registration document, as described in Part III, is required."

Dr. Gottesman noted that entry 27 of Appendix E of the Guidelines currently permits cloning of DNA among members of the genus into nonpathogenic Streptomyces under P1 containment conditions. She said the request would extend

this by eliminating the requirement for a registration document. She felt that this action would be premature; the working group evaluating the status of the Guidelines will be considering the need for registration documents in general. She moved to reject the request. Dr. Fedoroff seconded the motion. By a vote of fifteen in favor, none opposed, and three abstentions, RAC rejected Dr. Cohen's request.

XVI. PROPOSED LARGE SCALE EXPERIMENTS

Dr. Pinon initiated the review of a proposal (tab 1001) from Dr. Barry Nall of the University of Texas. Dr. Nall requested permission to perform large-scale fermentations of Saccharomyces cerevisiae strains containing recombinant DNA plasmids. The recombinant plasmid vectors consist of DNA from the E. coli plasmid pBR322, the yeast 2 micron circle, and yeast chromosomal DNA. The Saccharomyces cerevisiae cytochrome c gene will be ligated into these plasmid vectors. Dr. Pinon said the experiment is essentially self-cloning, and the sequences to be cloned are well characterized. He noted that a registration document had not been submitted and suggested approval at P1-LS containment be contingent upon submission of this document. He so moved. Dr. Williams seconded the motion. Dr. Gottesman felt P1-LS containment was not necessary and offered an amendment to substitute the words "good microbiological practice" for P1-LS. Dr. McKinney disagreed, he felt P1-LS actually represented "good microbiological practice." Mr. Thornton called the vote on Dr. Gottesman's proposed amendment. By a vote of four in favor, thirteen opposed, and three abstentions, the RAC refused the proposed amendment. Mr. Thornton then called the vote on Dr. Pinon's motion to approve the request at the P1-LS level of containment.

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

Mr. Thornton then called on Dr. Campbell to begin review of the proposal (tab 1018) from Dr. Hamilton O. Smith of the Johns Hopkins University. Dr. Smith requested permission to grow large quantities of the Hha II restriction and modification genes from Haemophilus haemolyticus, in E. coli. Dr. Campbell said the DNA to be cloned in a pBR322 vector is a reasonably small, well-defined segment. He moved to approve cloning of the plasmid in E. coli at the P1-LS level of containment. By a vote of nineteen in favor, none opposed, and no abstentions, the RAC adopted the motion.

XVII. REQUEST FOR APPROVAL OF CERTAIN EXPERIMENTS INVOLVING ANABAENA AND NOSTOC

Dr. Fedoroff introduced the proposal (tab 1013) of Dr. C. Peter Wolk of Michigan State University. Dr. Wolk requested permission to (a) construct a recombinant molecule from an E. coli plasmid and DNA from a strain of Anabaena, (b) propagate that DNA in an E. coli K-12 strain, and (c) transfer the cloned DNA to a different Anabaena strain. DNA may also be derived from and/or transferred to strains of the closely related genus, Nostoc.

Dr. Fedoroff said the transfer of this recombinant DNA into Anabaena or Nostoc is currently permitted, under P3 containment conditions, by Section III-O-2 of the Guidelines. Dr. Wolk requested a lowering of containment to Pl. Dr. Fedoroff said the request was internally inconsistent in that Dr. Wolk states that no cyanobacteria are known or suspected pathogens; however, the supporting documentation indicates that these organisms do produce toxins. She moved that RAC approve the request contingent upon ORDA receiving documentation that the strains Dr. Wolk uses will not be toxin producers.

Drs. Berns, McGarrity and McKinney felt it inappropriate to approve an incomplete, inconsistent proposal. Dr. Fedoroff withdrew her original motion, and then moved disapproval of the proposal with the request that the principal investigator submit a clearer and internally consistent proposal. By a vote of nineteen in favor, none opposed, and no abstentions, the RAC adopted the motion.

XVIII. GUIDELINES FOR RECOMBINANT DNA EXPERIMENTS WITH GENES CODING FOR TOXINS

Dr. Maas initiated the discussion of the proposed guidelines for recombinant DNA experiments with genes coding for toxins (tabs 996, 997, 1015/VIII). Dr. Maas stated that an ad hoc working group had been constituted several months ago to attempt to evaluate Section I-D-2 of the Guidelines which deals with potent toxins. The working group was composed of Drs. Werner Maas and Alan Bernheimer of New York University, Dr. John Collier of Yale University, Dr. Susan Gottesman of the NIH, Dr. Michael Gill of Tufts University, Dr. Myron Levine of the University of Maryland, and Dr. James Mason of the Utah State Department of Health. He said that the group evaluated toxins as pharmacological agents per se without consideration of other characteristics of the organism that produces the toxin.

Dr. Novick, citing recent research showing that a toxin produced by a Bacillus is functionally expressed in E. coli host-vector systems, said that the document is timely.

Dr. Levine pointed out that important vaccine development is dependent on recombinant DNA manipulations. He said the proposal under consideration will advance work in this area by clarifying the status of toxins under the Guidelines. He then deferred to Dr. Gill, an ad hoc consultant, who had been instrumental in constructing the proposal on toxins.

Dr. Gill called the committee's attention to tab 1015/VIII in the Federal Register. The proposal would modify Section I-D-2 of the Prohibitions and would add a new Appendix G to the Guidelines. Containment conditions are assigned for cloning toxins in E. coli K-12. A principal investigator wishing to use other host-vector systems would have to contact ORDA which will consult with the ad hoc working group on toxins. Toxins were divided into four groups on the basis of potency. Cloning of DNA coding for toxins with an LD₅₀ of less than 100 nanograms per kilogram body weight is prohibited; cloning of genes coding for toxins with an LD₅₀ of 100 nanograms

to 1 microgram per kilogram may be performed at P3 + EK1 or P2 + EK2; cloning of DNAs coding for toxins with an LD₅₀ of 1 microgram to 100 micrograms per kilogram may be performed at P1 + EK1. P1 + EK1 containment may be used for specified enterotoxins. DNAs coding for proteins with an LD₅₀ of greater than 100 micrograms per kilogram may be handled as nontoxins under the Guidelines.

Dr. Gill said the effects of intrainstestinal production of cytotoxic toxins might include (1) direct damage to the intestinal lining and (2) passage through the lining into the bloodstream. In the absence of information on the effect of *E. coli* elaborating toxin in the human intestine, the working group based the classification on available data from humans, primates, and small animals, generated by intravenous or parenteral administration of toxins. A listing of toxins by potency has been prepared and is available from ORDA. In addition, the working group outlined a procedure for evaluating toxins to be added to the list in the future. The procedure is also available from ORDA.

Dr. Gill outlined the types of data used to determine toxicity:

- (1) Human toxicity, if known, would be paramount in fixing containment levels.
- (2) If human toxicity is not known, it would be inferred from assays of toxicity to other primates.
- (3) If neither human nor primate toxicity is known, it would be inferred from the LD₅₀ of the most sensitive of three small animal species (mice, guinea pigs and rabbits).

Dr. Gill said that in those cases in which there is human data, man is not significantly more sensitive to the toxin than the most sensitive of three small animal species (mouse, rabbit or guinea pig).

Dr. Gill pointed out to RAC that diphtheria toxin appears to have an LD₅₀ of 100 nanograms or less per kilogram body weight in humans. The working group designated P3 + EK1 containment, but Dr. Gill asked RAC to consider whether this containment was appropriate.

Dr. Levine reiterated that although the proposed classification is based on pharmacological potency, the toxin delivery system is highly important to the pathogenicity of a toxin producing organism in nature.

Dr. Nightingale asked if the toxins prohibited by the proposed classification (botulinum, tetanus, Shigella neurotoxin) would be the only prohibited toxins. Dr. Gill replied that these toxins are the only ones currently known to have an LD₅₀ of 100 nanograms or less per kilogram (other than diphtheria which is right on the borderline). If other toxins are found in the future to have an LD₅₀ in this range, they would be put on the list and the cloning of their gene would be prohibited.

Dr. Laird argued against prohibiting research on tetanus and botulism toxins. He said it is highly important to develop vaccines for these toxins. Dr. Gottesman said that while those toxins are placed in the prohibited category, the prohibition is not absolute, individuals may come to RAC with a case-by-case request for an exception to any prohibition.

Dr. Gottesman asked Dr. Gill which route of delivery elicits the most sensitive enterotoxin response. Dr. Gill replied that the enteral route was most effective. These toxins are the only toxins which are more toxic when administered enterally than parenterally. Dr. Gottesman asked where cholera toxin would fall in the proposed classification on the basis of enteral and parenteral LD₅₀s. Dr. Gill replied that cholera toxin would not be considered a toxin under the proposed classification when administered parenterally. The LD₅₀ of cholera toxin administered enterally, would place it under Section 2-b of the proposed Appendix G, were there no Section 2-c.

Dr. Holmes praised the proposal presented by the working group but expressed several reservations. He said the premise that humans will be as sensitive as the most sensitive of three small animal species is based on data for seven toxins, as information on human toxicity for most toxins is not available. This assumption may not be true for all toxins. He was also concerned with the question of creating new ecological niches. Staphylococcal enterotoxin F, implicated in toxic shock syndrome, is not highly toxic, yet we suddenly have the appearance of this new clinically important syndrome. He questioned whether toxin producing recombinant organisms able to survive in other sites, such as vagina, respiratory tract, or wounds, might be highly hazardous. He felt additional data should be generated to address these questions. He suggested that a procedure involving case-by-case evaluation, at least at some level, as with the specific proposal involving diphtheria toxin to be reviewed by the RAC later in the meeting, was appropriate until more information was gathered.

Dr. Nightingale questioned the wisdom of discussing treatability in setting containment levels for the enterotoxins. Timing, availability of treatment, etc., affect the outcome of treatment. Drs. McGarrity and Goldstein also objected to the concept that physical containment conditions need not be as stringent if physiological remedies exist. Dr. Nightingale noted that toxins whose end point is not immediate death, such as those which cause cancer years later, are not included in the classification.

Dr. Nightingale proposed that the classification of toxins be considered in context of the upcoming total review of the Guidelines. She moved to refer consideration of the document to the working group for revision of the Guidelines. Dr. Goldstein seconded the motion.

Dr. Levine opposed the motion. He said that the classification generated by the ad hoc working group on toxins represented six months of work by expert toxicologists. The issue presented enormous challenges in reviewing available data, and in constructing a proposal acceptable to all members of the working group. The proposed language is the working group's best

effort to come to grips with balancing potential risk versus potential benefit. He pointed out that virtually all of the points raised by RAC had been discussed and carefully evaluated by the working group. The format based on pharmacological criteria is an extremely conservative approach since many factors other than the toxin itself are of great consequence in pathogenicity. Dr. Maas supported Dr. Levine's statement. The proposal is conservative; it clarifies the issues and distinguishes between potent and nonpotent toxins. He offered a substitute motion to accept the proposal as it appeared in the Federal Register with the provision that these restrictions override all other sections of the Guidelines, e.g., exemptions, self-cloning, etc. Dr. Fedoroff seconded the motion.

Dr. Gottesman offered the following amendments to the proposal as it appeared in the Federal Register:

- (1) The words "Pl + EK1" will be substituted for the words "Section III-O."
- (2) Language indicating that these specifications will override other sections of the Guidelines, e.g., "exemptions" and "return to host of origin" will be added to Section I-D-2 of the Guidelines.
- (3) Language describing treatability of enterotoxin effects will be deleted.

Dr. Maas accepted the proposed amendments.

Dr. Berns said he supported the proposed language in a general sense, but felt RAC must monitor toxin experiments. He said he would support the proposal if language requiring registration of toxin experiments with ORDA was incorporated.

Dr. Maas accepted this suggestion as an amendment. Dr. Adelberg urged the committee to support Dr. Maas' amended motion, as he felt Dr. Nightingale's proposal would simply postpone the discussion. Dr. McKinney also urged the committee to accept the proposal. Dr. Fedoroff complimented the working group for an extremely thoughtful, thorough treatment of a very difficult subject. Dr. Nightingale said that she supported the substitute motion.

Dr. Gill suggested a clarification in the proposed language in regard to registration with ORDA; he suggested the sentence "Experiments involving toxins that are lethal at 100 micrograms or less shall be registered with ORDA" be added to Section 1, General Information, of proposed Appendix G. Dr. Gottesman agreed, as did Dr. Maas. Dr. Williams called for the vote on the question. By a vote of fourteen in favor, three opposed, and one abstention, the call for the question carried. Mr. Thornton then called the vote on Dr. Maas' substitute motion as amended. This was to substitute the motion proposed by Dr. Maas, i.e., to accept the language in the Federal Register (1015/VIII) with four changes:

- (1) "Section III-0" to be changed to "Pl-EK1" in Sections 2-b and 2-c of Appendix G.
- (2) The introductory text of Section 2-c of Appendix G dealing with treatability of enterotoxin effects to be eliminated.
- (3) Language to be added at the end of Section I-D-2 of the Guidelines indicating that Appendix G specifications override other specifications of the Guidelines (e.g., exemptions or return to host of origin experiments).
- (4) Language to be added to Section 1 of Appendix G that experiments involving toxins that are lethal at 100 micrograms or less shall be registered with ORDA.

By a vote of sixteen in favor, three opposed, and no abstentions the RAC adopted the substitute motion in place of Dr. Nightingale's previous motion to defer consideration.

Dr. Novick asked if he might offer an amendment at this point. Mr. Thornton said that technically he could rule no amendment was in order at this point in the voting process; but in an effort to permit all points of view to be heard, he would recognize Dr. Novick. Dr. Novick said he wished to add the word "prior" to the added sentence specifying registration with ORDA. Dr. Maas accepted Dr. Novick's amendment. Mr. Thornton then called the vote on the motion, i.e., to accept the language in the Federal Register (tab 1015/VIII) with the four changes listed above including the word "prior" in the fourth change. The RAC adopted the modified language by a vote of eighteen in favor, none opposed, and one abstention. Mr. Thornton expressed his appreciation to all involved in preparing the proposal.

Dr. Gill then summarized the risk assessment experiments proposed by the toxin working group (Attachment II). Mr. Thornton said he intended to refer the proposal to the Risk Assessment Subcommittee for further consideration.

XIX. REQUEST TO CLONE THE VIBRIO CHOLERAE ENTEROTOXIN GENE

Dr. Holmes initiated discussion on the proposal (tabs 1002, 1010, 1015/X) submitted by Dr. John Mekalanos of Harvard Medical School. Dr. Mekalanos requested an exemption from Section I-D-2 of the Guidelines, which prohibits the formation of recombinant DNAs containing genes for the biosynthesis of toxins potent for vertebrates. Dr. Mekalanos wishes to clone the Vibrio cholerae sequence coding for the biosynthesis of cholera toxin. Dr. Mekalanos requested consideration of the appropriate level to perform three experiments in E. coli K-12: to clone sequences coding for the (1) LT A subunit, (2) the LT B subunit, and (3) the LT A and LT B subunits. Dr. Holmes said that Dr. Mekalanos suggested experiments 1 and 2 might be performed under P2 + EK1 containment; no active LT toxin will be synthesized. Dr. Holmes said the toxin classification RAC had just adopted would indicate

P1 + EK1 containment for this type of experiment. He found P1 + EK1 adequate for experiments 1 and 2. Dr. Holmes said Dr. Mekalanos had requested P2 + EK1 containment for experiment 3, but would be ready to use P2 + EK2. Dr. Holmes felt experiment 3 could be performed under P1 + EK1 conditions as specified by the recently adopted toxin classification.

Dr. Levine viewed the request as important work. He agreed with Dr. Holmes that P1 + EK1 containment conditions were appropriate for all three experiments. Dr. Holmes moved approval of experiments 1, 2, and 3 at the P1 + EK1 level of containment. Dr. Levine seconded the motion.

Dr. Novick suggested that P2 physical containment might be more appropriate to the experiments; he expressed concern that colonization factors, permitting E. coli to attach to small bowel adhesion sites, might be picked up by E. coli making cholera toxin. Dr. Levine said recent data have demonstrated that colonization factors alone will not create a pathogen. Dr. Novick moved an amendment to require the experiments to be performed under P2 containment conditions. He did not view P2 containment as an overwhelming burden and felt P2 would alleviate residual anxieties. Dr. Holmes said he would accept P2 requirements for experiment 3 but felt P1 + EK1 was adequate for experiments 1 and 2.

Dr. Levine replied that P2 containment would afford little or no additional safety since Vibrio cholerae is not spread by the aerosol route. As control of aerosolization is the most significant distinction between P1 and P2 containment, he felt P1 specifications are adequate to contain the experiments. He also pointed out that the general classification of toxins just approved by the RAC permits the experiments at P1 + EK1.

Dr. Holmes said he wished to rephrase his motion to make containment conditions contingent on the Director's decision concerning the general toxin classification (Appendix G); if Dr. Fredrickson accepts the general proposal, Dr. Mekalanos may proceed with experiments 1, 2, and 3 under P1 containment conditions. Should Dr. Fredrickson not accept the proposal, the RAC recommends that Dr. Mekalanos may perform experiments 1 and 2 under P1 conditions, but must use P2 containment for experiment 3. Dr. Levine, who had seconded Dr. Holmes' original motion, did not accept the modified language; Mr. Thornton called the vote on the modified language. By a vote of three in favor, ten opposed, and three abstentions, the RAC refused the modified language. Mr. Thornton then called the vote on Dr. Holmes' original language, i.e., approval of the proposed experiments at P1 + EK1 containment. By a vote of fifteen in favor, none opposed, and one abstention, the RAC adopted the motion.

XX. CLONING AND EXPRESSION OF DNA CODING FOR DIPHTHERIA TOXIN

Dr. Holmes introduced the request (tab 1003, 1015/IX) of Dr. John Murphy of Harvard University. Dr. Murphy proposed to clone in E. coli K-12 the 3.9 kb Bam restriction fragment of Coryneophage Beta which carries the

diphtheria toxin structural gene. Dr. Murphy proposed to use P4 containment and to perform the experiments at Fort Detrick. If P3 containment were permitted, he would propose to perform the experiments at Sidney Farber Cancer Center in Boston or at Harvard University in Cambridge, Massachusetts. Dr. Murphy would perform risk assessment experiments, and would study toxin secretion, localization in E. coli, and the entry of toxin into eukaryotic cells.

Dr. Levine spoke of one potential future benefit of the proposed research: hormones that home to specific organs, might be linked to portions of the diphtheria toxin and the combination molecules might serve as highly specific agents in cancer chemotherapy. Dr. Levine strongly supported that portion of the request dealing with possible risk assessment studies. He noted that P3 containment conditions are specified by the general toxin classification, previously adopted by RAC; and he felt P3 was adequate containment.

Dr. Gottesman asked Dr. Gill to review the data on the potency of diphtheria toxin, which had led to its position in Appendix G in the class allowed at P3 + EK1 containment. Dr. Gill said the LD₅₀ of diphtheria toxin, in the most sensitive small animal (guinea pig), is 160 nanograms per kilogram body weight. The LD₅₀ in humans is estimated to be equal to or less than 100 nanograms per kilogram body weight. This figure was extrapolated from an incident in Japan in which children were inadvertently injected with diphtheria toxin rather than diphtheria toxoid. It thus falls close to the borderline of 100 nanograms per kilogram body weight, which would separate a toxin the cloning of whose gene would be prohibited from a toxin the cloning of whose gene would be allowed at P3 + EK1 in Appendix G. Dr. Gottesman said that she would support P4 containment for the proposed experiment. Dr. Levine said he could support P4 containment, but questioned whether this might conflict with the action taken earlier in the meeting on the general toxin classification. Dr. Talbot said that Dr. Fredrickson could resolve any conflict when he promulgates his decision on the recommendations from this meeting.

Dr. Levine moved acceptance of the proposal at P4 containment. Dr. Goldstein seconded the motion. Be a vote of fifteen in favor, none opposed, and one abstention, the RAC adopted the motion.

Dr. Levine suggested that if the genes are successfully cloned, an E. coli host-vector system containing the plasmid and capable of colonizing a pig might be used in an additional risk assessment experiment. The pigs should be colonized under P4 containment conditions. Dr. Murphy expressed interest in pursuing such experiments, but it was pointed out that it may not be possible to introduce a pig into the P4 line.

XXI. REQUEST TO CLONE THE GENETIC DETERMINANT OF THE TOXIC-SHOCK SYNDROME CAUSED BY STAPHYLOCOCCUS AUREUS

Dr. Mason initiated the review of a proposal (tabs 1008, 1015/XI) from Dr. Richard Novick of the Public Health Research Institute of the City of New York, Inc. Dr. Novick requested permission to clone in Staphylococcus aureus the genetic determinant of the toxic-shock syndrome caused by S. aureus. Dr. Novick stated that recombinant DNA techniques promise the most rapid and direct means of unraveling the biology of the determinant and of the disease. He requested permission to conduct the experiments under P2 containment using S. aureus strain RN 450, which is non-hemolytic, non-pigmented, and lacking any detectable prophage. Dr. Mason said he felt the work should be done but said containment should probably be assessed at the P3 level. Dr. Levine said the proposal was not sufficiently amplified; too little data had been submitted to permit a fair appraisal. Dr. Holmes agreed, he said he would like to examine additional information on the toxin and on the enfeebled S. aureus strain to be used as a host.

Dr. Novick noted that the RAC at a previous meeting had approved the cloning of the S. aureus ToxA gene in B. subtilis at P3. He admitted that the exact cause of the syndrome has not been elucidated and said he hoped to identify the toxin's role through these studies.

Dr. Nightingale said the presentation was not well-prepared. She felt that the information available on toxic-shock syndrome may not be sufficient to permit a reasonable appraisal of the proposed experiments. She requested additional data.

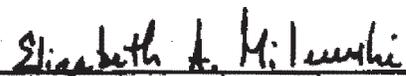
Dr. Maas said that currently there is a state of confusion as to which toxin is the cause of toxic shock syndrome. He felt the issues should be clarified before permission is given to begin cloning the toxin gene.

Dr. Gottesman said the previous RAC approval to clone the S. aureus Tox A gene stipulated P3 containment conditions with an HV2 Bacillus subtilis host-vector system. She questioned whether S. aureus would be a safer host-vector system than an HV2 B. subtilis host-vector system. The toxin's capacity to potentiate other toxins is also an issue. Dr. Novick said he would withdraw his proposal and attempt to provide better documentation at a future time.

XXII. ADJOURNMENT

The meeting was adjourned at 1:25 p.m. on April 24, 1981.

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 Attachments are accurate and complete.

September 10, 1981
Date



Ray Thornton, J.D.
Chairman
Recombinant DNA Advisory Committee