

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

RECOMBINANT DNA ADVISORY COMMITTEE

MINUTES OF MEETING

FEBRUARY 8-9, 1982

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MINUTES OF MEETING<sup>1</sup>

FEBRUARY 8-9, 1982

The Recombinant DNA Advisory Committee (RAC) was convened for its twenty-fourth meeting at 9:00 a.m. on February 8, 1982, at the Marriott Hotel, Salon D and E, 5151 Pooks Hill Road, Bethesda, Maryland 20814. Mr. Ray Thornton (Chairman), President, Arkansas State University, presided. In accordance with Public Law 92-463, the meeting was open to the public.

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

Abdul Karim Ahmed; David Baltimore; Kenneth Berns; L. Albert Daloz; Nina Fedoroff; David Friedman; Richard Goldstein; King Holmes; Patricia King; Arthur Landy; Myron Levine; Werner Maas; David Martin; James Mason; Gerard McGarrity; Robert McKinney; Robert Mitchell; Elena Nightingale; Ramon Pinon; Mark Saginor; John Scandalios; and William J. Gartland, Jr., Executive Secretary.

A Committee roster is attached. (Attachment I)

The following ad hoc consultant to the Committee was present:

Susan K. Gottesman, National Institutes of Health.

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

Howard Berman, U.S. Veterans Administration; Chia T. Chen, OSHA, U.S. Department of Labor; George Duda, Department of Energy; Timothy J. Henry, Food and Drug Administration; Herman Lewis, National Science Foundation; Henry Miller, Bureau of Drugs, FDA; Sue Tolin, U.S. Department of Agriculture; and William J. Walsh, III, U.S. Department of State.

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<sup>1</sup>The 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.

Other National Institutes of Health staff present were:

Robert C. Backus, OD; Stanley Barban, NIAID; W. Emmett Barkley, OD; Becky Connors, NIAID; Irving Delappe, NIAID; Joan Hartman, NIAID; Elizabeth Milewski, NIAID; Stanley Nagle, NIAID; Donald Ralbovsky, OD; Monica Schaeffer, OD; Robert Schreiber, NIAID; and Bernard Talbot, NIAID.

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

Beth Barban; Claudia Baskin, PMA Newsletter; Robert Bazell, NBC News; Tineke Bodde, BioScience Magazine; Michael Borisov, USSR Embassy; Irene Brandt, Eli Lilly & Company; Allan Buchanan, President's Commission on Medical Ethics; Dennis Cheek, University of Baltimore; Marc Collett, Molecular Genetics, Inc.; David Collins, Department of Justice; David Dickson, Nature; James Dougherty, National Endowment for the Humanities; Paula Dwyer, McGraw Hill; Larry Elliott, National Institute for Occupational Safety and Health; John Ferrugia, CBS; Shelly Fabares; Sam Fleming, Maver School; Jeffrey Fox, Chemical and Engineering News; John Galet, Schering Plough Corporation; Charles Gauth, Bethesda Research Laboratories; Lowell Harmison, Office of Assistant Secretary for Health; Clayton Hathaway, Monsanto Company; Judith Hautala, Genex Corporation; Pamela Haynes, Government Research Corporation; T. M. Helscher, Monsanto Company; Philip Hilts, Washington Post; William Huhn, Pfizer, Inc.; Dorothy Jessop, U.S. Department of Agriculture; Irving Johnson, Eli Lilly and Company; Judith A. Johnson, Library of Congress; M. J. Johnson, Pall Corporation; Roger Johnson, Genetic Engineering Letter; Eric Juengst, National Endowment for the Humanities; Neil Jurinski, NuChem Co, Inc.; James Kaper, University of Maryland; Geoffrey Karny, Office of Technology Assessment; Rihito Kimura, Georgetown University; Warren Leary, Associated Press; S. Edward Lee, Hoffman LaRoche, Inc.; Carter Leonard, Blue Sheet; W. Lepkowski, Chemical and Engineering News; Morris A. Levin, Environmental Protection Agency; Dan Liberman, Massachusetts Institute of Technology; Charles Marwick, New Scientist; Vincent Mazzola, U.S. Department of Agriculture; James McCullough, Library of Congress; Julia Miller, Science News; Bernard Mlynczak, Monsanto Company; Claire Nader; Norine Noonan, Science and Technology Committee, House of Representatives; Stephen Pijar, Food and Drug Administration; William Pilacinski, Molecular Genetics, Inc; Michael Pimentel, University of Maryland; Harvey Price, Industrial Biotechnology Association; Daniel Rift, Princeton University; Sheila Rosenthal, Environmental Protection Agency; Sandra Ronspies, Genentech, Inc.; Perc Reeve, American Cyanamid; Renie Schapiro, President's Commission on Medical Ethics; Harold Schmeck, New York Times; Stephanie Soucek, National Institute for Occupational Safety and Health; Marjory Sun, Science Magazine; Keith Swain, New England Nuclear; Ane Talbot; Terry Vass, Genentech, Inc.; Jonathan Weiswasser, Maver School; Susan Wright, University of Michigan; and Eileen Zalisk, NOVA.

## I. CALL TO ORDER AND OPENING REMARKS

Mr. Ray Thornton, Chairman, called the meeting to order at 9:00 a.m., on February 8, 1982. He introduced two new members of the Recombinant DNA Advisory Committee: Dr. David Friedman, Professor of Microbiology at the University of Michigan and Dr. David Martin, Professor of Medicine and Chief of Medical Genetics at the University of California Medical Center, San Francisco.

## II. MINUTES OF THE SEPTEMBER 10-11, 1981, MEETING

Mr. Thornton asked Dr. McGarrity to comment on the minutes (tab 1061) of the September 10-11, 1981, meeting. Dr. McGarrity said the minutes accurately reflected the September meeting, and moved that they be accepted. Dr. Fedoroff seconded the motion. Dr. McKinney requested a clarification of the language in Section XVI, Containment Conditions for Cloning and Expression of DNA Coding for Diphtheria Toxin. He suggested the language should be clarified to read:

"Dr. McKinney suggested RAC specify that the work be conducted in P3 laboratories in Building 550 of the Frederick Cancer Research Center under conditions specified by the local IBC."

Mr. Thornton called the question on the motion to accept the minutes with the clarified language. The motion was unanimously accepted.

## III. RISK ASSESSMENT STUDIES

Mr. Thornton invited Dr. Levine to present the summary of recombinant DNA risk assessment studies at tab 1057. Dr. Levine said that from the early days of recombinant DNA technology there has been concern about measures used to contain genetic recombinants. Sophisticated physical containment facilities can provide containment, however, such facilities are expensive to construct and to maintain. On the other hand, a degree of biological containment can be obtained, inexpensively, by selecting "safe" poorly mobilizable plasmids as cloning vectors and by using as hosts bacterial strains that do not colonize the human intestine.

The degree to which poorly mobilizable "safe" plasmids can or cannot be transferred from bacterium to bacterium within the human intestinal milieu is a critical assessment of containability. The Falmouth Conference on Recombinant DNA in 1977 formally addressed the question of plasmid mobilizability; the conferees recommended that risk assessment studies, consisting of feeding human volunteers E. coli K-12 with various plasmids, be performed. In 1979, an ad hoc Working Group for Risk Assessment was convened at NIH. At that meeting, experts reviewed the Falmouth protocol and pointed out that it would not be feasible to evaluate plasmid transfer using E. coli K-12 as the host, since E. coli K-12 does not colonize the human intestine and is rather rapidly eliminated from the bowel. An E. coli K-12 strain

would, thus, never reach high enough numbers in the human intestine for plasmid transfer to be detected if it occurred at low probability. The ad hoc Working Group suggested instead that the plasmids should be evaluated for mobilizability using as host an E. coli strain that readily colonizes the human intestine.

Dr. Levine said the risk assessment studies he would describe used E. coli strain HS-4 feeding studies to evaluate plasmid mobilizability. He said the study was to determine: (a) how well E. coli HS-4 colonized the human intestine; (b) whether indigenous coliforms would continue to co-habitate in the colon with HS-4; (c) whether and with what frequency a mobilizable plasmid would be transferred by triple crosses in vivo from HS-4 into indigenous coliforms; (d) whether and with what frequency a poorly mobilizable plasmid would be transferred by triple crosses in vivo from HS-4 into indigenous coliforms; and (e) whether and with what frequency a poorly mobilizable plasmid would be transferred in vivo, in the presence of a highly conjugative plasmid, from HS-4 into indigenous coliforms.

Dr. Levine said the experiments show that: (a) E. coli HS-4 very effectively colonizes the human intestine; (b) indigenous coliforms continue to cohabit the colon with HS-4 in most individuals; (c) mobilizable plasmids are transferred by triple crosses in vivo from HS-4 into indigenous coliforms; (d) a poorly mobilizable plasmid is not detectably transferred by triple crosses in vivo from HS-4 into indigenous coliforms; and (e) a poorly mobilizable plasmid transfers in the presence of a highly conjugative plasmid in vivo from HS-4 into indigenous coliforms.

Dr. Levine emphasized that experiment (d) described above examining whether a poorly mobilizable plasmid can be transferred in vivo by triple cross is the critical risk assessment study as it most resembles the potential laboratory accident. He pointed out, however, that very large numbers of organisms, in sodium bicarbonate to neutralize stomach acid, were fed to volunteers taking the antibiotic, tetracycline. The use of antacids and antibiotics is forbidden in a recombinant DNA laboratory, and the numbers of organisms administered in the study are unrealistically large in terms of what might occur in a laboratory accident. Even under these unrealistic conditions which enhance the possibility of transmission, there was no demonstrable transfer of the poorly mobilizable plasmid.

Dr. Levine said Dr. Stuart Levy of Tufts University had also performed feeding studies. Dr. Levine said Dr. Levy fed volunteers a debilitated E. coli K-12 strain containing a poorly mobilizable plasmid and two derepressed conjugative plasmids. The strain does not colonize, and no transfer of the plasmid to indigenous coliforms was observed. Dr. Levy's study, however, is more realistic in that it tests for plasmid transfer with a host-vector system actually used in recombinant DNA research. Dr. Martin asked if comparable studies had been performed in mice. Dr. Levine said that E. coli is not a major flora in the mouse and doesn't reach anywhere near the concentration per gram of fecal material that one gets in man; Dr. Levine felt that human feeding studies are the critical experiments.

#### IV. PROPOSED REVISION OF THE GUIDELINES

Mr. Thornton called the attention of the RAC to the major topic of the February 8-9, 1982, meeting, a discussion of two proposals (tabs 1050, 1056A, 1056B, 1056C/1, 1056C/7, 1056D, 1056E, 1056F, 1056G) to modify the the current NIH Guidelines for Research Involving Recombinant DNA Molecules.

Mr. Thornton said he would take a moment to give his personal perspective prior to resuming the role as committee chairman. He said former NIH Director, Donald Fredrickson, summarized the purposes of the Guidelines as (1) to establish a rapid, complete means of communication, (2) to assure that the Guidelines are conservative yet allow research to proceed, and (3) to permit public participation in the formulation of public policy. Mr. Thornton noted the difficulty of establishing and maintaining communication between public policy decision makers and experts in a scientific field. NIH has devised a mechanism which successfully maintains this communication, and he would not wish to abandon it.

Mr. Thornton then described the Guidelines from a lawyer's perspective. He noted that the Guidelines are not laws; he thought this is good since laws are difficult to formulate and difficult to change. Neither are they regulations; regulations are subject to formal revision procedures much more rigid than those RAC and the NIH follow in modifying the Guidelines. Neither are the Guidelines simply statements of good practice. The RAC and the NIH have been responsive to change, not as quickly perhaps as some would have preferred, but quickly enough that the advance of science has not been significantly impeded.

Mr. Thornton then recognized Dr. Baltimore who referred to the December 4, 1981, proposal which RAC had recommended for publication in the Federal Register (46 FR 59368). Dr. Baltimore said that the proposal had elicited tremendous response. He said that conversion to a voluntary code of standard practice, as described in the December 4, 1981, Federal Register, is appropriate. Although the current NIH Guidelines are not formal regulations, they have instituted an informal regulatory process. He expressed hope that the philosophy of voluntary compliance expressed in the December 4, 1981, proposal would be accepted.

Dr. Baltimore suggested that some of the concerns expressed about the December 4, 1981, proposal by correspondents could be addressed and met by modifications. Some correspondents had expressed concern that the IBCs would be dismantled. Dr. Baltimore assumed that with the language of the December 4, 1981, proposal, the IBCs would remain in place. He said he had, however, prepared an amendment, which might be added during the discussion, specifying a continuing role for IBCs.

Dr. Baltimore said that in setting P1 containment conditions, the December 4, 1981, proposal implies there could not be deliberate release of recombinant organisms into the environment. It is clear from the letters received in response to the proposal, however, that some people would prefer an

explicit statement to that effect. Dr. Baltimore said that if RAC felt it was necessary, he would support an amendment to the December 4, 1981, proposal to accomplish that aim.

Finally, Dr. Baltimore suggested the language of Section I-A might be modified to include a strong statement that although voluntary, adherence to the Guidelines is strongly recommended. He said the December 4, 1981, proposal with these amendments would be responsive to comments received. He then moved the proposal appearing in the December 4, 1981, Federal Register (46 FR 59368) as an item for discussion. The motion was seconded by Dr. McGarrity.

Dr. Baltimore made an additional statement in response to certain written comments received. He said that he has never hidden his affiliation with the company, Collaborative Research, of Waltham, Massachusetts. He stressed, however, that if he were acting for the company, he would not be supporting the December 4, 1981, proposal because he said it is not in the interests of any institution in the Boston area, as it might lead to more stringent regulation at the local level. He said he supported the December 4, 1981, proposal because he believes it is correct.

Dr. Nightingale said that letters commenting on the proposals indicate many remaining concerns in both the scientific and public sectors. In her view, these concerns are not adequately addressed by the December 4, 1981, proposal even if that proposal were modified as just suggested by Dr. Baltimore.

Dr. Nightingale said that there is not a clear consensus for eliminating the mandatory nature of the Guidelines or eliminating the requirement for IBCs. She said the issue of scale-up needs further discussion. She expressed the belief that removing the mandatory nature of the Guidelines would stimulate a variety of legislative actions across the country, possibly resulting in regulatory variation from location to location. She also suggested that although the probability of an event with disastrous consequences is very small, one must acknowledge that gaps in scientific knowledge exist; if such a very rare event should occur, there could be tremendous backlash against the scientific community.

Dr. Nightingale said the December 7, 1981 (46 FR 59734, Part 7, "Gottesman"), proposal would simplify the Guidelines and remove many restrictions. Dr. Nightingale said she had a list of at least six ways in which the Gottesman proposal could be further simplified, and restrictions further removed, by the next RAC meeting. Dr. Nightingale then moved acceptance of the December 7, 1981, "Gottesman" proposal as a substitute motion with a commitment to continue to review, reorganize, simplify, and remove restrictions from the Guidelines as expeditiously as possible. Dr. Fedoroff seconded the motion.

Dr. Berns said that the current Guidelines are cumbersome and complex. The RAC has several options. The most significant issue is the mandatory nature of the Guidelines. He thought having IBCs is good, and recommended keeping the RAC. He stated a preference for readily understandable Guidelines.

Dr. Mason said RAC has acted responsibly in the process of reviewing the Guidelines. He supported the need for IBCs in both academia and industry. Indeed this type of activity should not be limited to the recombinant DNA field but should be encouraged generically. He feared that RAC, by its endorsement for publication of the December 4, 1981, proposal, did not convey to the public the importance of IBCs. Dr. Mason suggested that certain issues should be carefully scrutinized, including deliberate release of recombinant containing organisms into the environment and the cloning of genes for drug resistance and for certain toxins. Dr. Mason expressed the belief that the NIH Guidelines ultimately should and will become voluntary, but suggested they should remain mandatory for the time being for at least two reasons: (1) more information should be collected, particularly in regard to some of the areas currently prohibited; and (2) the public is not yet ready for voluntary guidelines.

Dr. Goldstein said that he could not support the December 4 proposal. He said that he supports the December 7 proposal as it simplifies the Guidelines, specifies IBCs, and maintains mandatory Guidelines. He stated that haphazard local regulations, varying from community to community, and hindering the research, will result if national oversight is not maintained. He felt the December 7, 1981, proposal does not deal adequately with large-scale work and that area should be reviewed.

Ms. King noted that at the September 8-9, 1981, RAC meeting she had not supported what became the December 4, 1981, proposal. She believes the December 7 "Gottesman" proposal is where the RAC should begin in trying to reach a final position. She expressed the belief that regulation is justified by concerns about safety. Arguments that recombinant DNA is no more dangerous than other forms of biomedical research have been advanced. This does not lead Ms. King to the conclusion that only a voluntary code of conduct is necessary. She suggested, rather, that if other research areas pose similar risk, then perhaps they too should be regulated. She favored mandatory Guidelines with sanctions and a monitoring system. The structure should not yet be dismantled nor should it be made voluntary. Otherwise a system of fragmented regulations at the state and local level might develop.

Mr. Thornton recognized Dr. Gottesman who had authored the December 7, 1981, proposal. Dr. Gottesman said her proposal is based on the assessment of risks in the document "Evaluation of the Risks Associated with Recombinant DNA" (46 FR 59385). She noted that that document had been generated by the Working Group on Revision of the Guidelines during the summer of 1981. On the basis of that evaluation, she had concluded that there are several types of experiments about which questions remain or about which so little is known that no absolute conclusion can be drawn. For these types of experiments she felt a mandatory record-keeping and oversight mechanism is appropriate.

Dr. Gottesman said her proposal requires RAC review and NIH approval for certain experiments involving toxin genes, drug resistance genes, and release into the environment. Responsibility for oversight of certain

other experiments is delegated to the IBCs. The types of experiments to be reviewed and IBC review procedures might be modified by RAC. RAC may wish to permit the IBCs greater leeway in lowering containment for certain experiments. Dr. Gottesman noted that her proposal does not alter the status of currently exempt experiments.

Mr. Daloz said that specialists in general tend to develop tunnel-vision so that their own concerns become uppermost in their minds. He noted that many laws and guidelines regulate our daily lives, and that even if the NIH Guidelines were eliminated, other agencies might institute guidelines or regulations. Mr. Daloz expressed his support for the December 7, 1981, proposal; he said, in any event, the IBCs should be retained.

Dr. McKinney said he had discussed the December 4 and December 7 proposals with scientists, lawyers, and representatives of commercial organizations. He said the researchers he had spoken with are approximately evenly divided in their support of mandatory vs. voluntary Guidelines. Regarding the current prohibitions, Dr. McKinney said many people felt certain experiments should be monitored and controlled.

Dr. McKinney said that previously the RAC had extricated itself from "regulating" large-scale activities. He felt the reintroduction of the question of how to oversee large-scale work was retrogressive; RAC should address science issues and avoid reviewing large-scale activities per se.

Finally, Dr. McKinney noted that some correspondents mentioned the negative effects the Guidelines have had on research. He said the committee must also take into account the beneficial aspects of the review process; in his view the benefits far outweigh any negative aspects. He said RAC would be remiss if it eliminated oversight over recombinant DNA research before more data are accumulated.

Mr. Mitchell said he had made a rough analysis of the opinions submitted by commentators on the proposals. According to his estimate, approximately half favored the December 4 proposal; the other half favored either the current Guidelines or modest changes therein, or the December 7 proposal.

Mr. Mitchell said the press gives the impression that the recombinant DNA field is advancing very rapidly. These accounts do not support the allegation that the Guidelines have inhibited research. He suggested that should the NIH change the Guidelines substantially, RAC would find itself in an untenable position; it would forfeit the opportunity to "move" the technology on a rational basis, and uniformity of standards would be lost. Mr. Mitchell suggested that adoption of the December 4, 1981, proposal would destroy some of the scientific community's credibility. He said that should Congress ever again consider national legislation, scientists could no longer argue they were following a policy of self-regulation.

Mr. Mitchell said he had attended a panel meeting of the California legislature's Committee on Health on December 14, 1981. He said these legislators, few of whom have a scientific background, spoke in terms of public perceptions. He questioned how many of those legislators would understand the scientific arguments or attempt to comprehend technical presentations.

Mr. Mitchell said he supported the December 7 proposal as it maintains the mandatory nature of the Guidelines and the requirement for IBCs. Dr. Fedoroff said she strongly supported the December 7 proposal and urged that a mechanism for further simplification be introduced.

Dr. Saginor said that the recombinant DNA issue could easily become a political football; the Guidelines have restrained politicians from using this as an issue. He added that the RAC as a central committee providing a forum for discussion is necessary. He supported the December 7, 1981, proposal.

Dr. Irving Johnson of Eli Lilly and Company said Eli Lilly had commented favorably on both the December 4 and the December 7 proposals, although he had reservations about both proposals. He said the December 4 proposal provides no "trackability". The December 7 proposal, while it simplifies the Guidelines, perpetuates unnecessary bookkeeping. He said that Eli Lilly and Company recommends mandatory retention of IBCs which should be required to report problems to the RAC.

Dr. Johnson pointed out that representatives of regulatory agencies are on the Interagency Recombinant DNA Committee and have liaison representatives to the RAC. These representatives are there to monitor events and suggest appropriate action to their agencies. For a company involved in interstate commerce such as Eli Lilly and Company, these agencies represent regulations which are mandatory and not voluntary.

Dr. Johnson said he had attended the November 1981 hearings of the California Legislature's Committee on Health and had detected little concern over risk at that hearing. Concerns were expressed, however, over moral and ethical problems. Dr. Johnson expressed concern about again raising the issue of large-scale work and cited the safety of large-scale equipment. He proposed amending the December 4, 1981, proposal to require retention of IBCs.

Dr. McGarrity said that he has concluded that recombinant DNA research presents no hazards beyond those normally associated with microbiological research. This is not to say there are no problems in other areas of biomedical research; however, these hazards have been adequately handled. He stated that it is time to stop the discriminatory treatment of recombinant DNA research. He favored the December 4, 1981, proposal with some modifications.

Dr. Holmes said he favored retaining mandatory Guidelines and the requirement for IBCs. He rejected the argument that recombinant DNA activities should not require oversight because other areas of microbiological or biomedical research do not have special oversight. He said he would support the December 7, 1981, proposal with the addition of a recommendation that IBCs also review non-recombinant DNA research that is similar to research covered by Section III of the Guidelines.

Dr. Baltimore reiterated his belief that recombinant DNA research is no more hazardous than experiments in the mainstream of biomedical research. He felt this was the judgement of a majority of the scientific community, and that the December 4, 1981, proposal reflects this consensus. He said fear of local regulation or fear of leaving industry with no code for legal protection were not reasons for maintaining mandatory Guidelines. Adoption of the December 4, 1981, proposal would send a message to States and localities that the RAC concludes that regulations are not necessary.

Finally, Dr. Baltimore said that the CDC "Classification of Etiological Agents on the Basis of Hazard" is not appropriate for use in classifying recombinant DNA experiments.

Dr. Lewis of the National Science Foundation suggested greater flexibility in IBC specifications might be desirable. Dr. Landy said that he supported the original Baltimore-Campbell proposal, and subsequently the December 4, 1981, proposal, as the only intellectually honest recognition of the relationship between the unestablished potential risk in recombinant DNA research and known risk in other areas of research which are not regulated. In attempting to rationalize support for greater controls over recombinant DNA research than over work with known pathogens, Dr. Landy said the training, procedures, and restraints applied by the select group of investigators studying pathogens would not necessarily have been followed by all those now using recombinant DNA techniques.

Dr. Gottesman concurred with Dr. Landy's rationalization and added that investigators studying pathogens know the properties of these organisms; recombinant organisms might express unexpected properties.

Dr. Maas said he saw no logic in having guidelines for one type of experimental procedure, which is rapidly becoming a very commonly employed technique, and having no regulations for other types of more dangerous procedures, such as work with chemical carcinogens.

Dr. Gottesman said that mandatory guidelines are not necessarily synonymous with bureaucracy. She noted that the December 7, 1981, proposal no longer requires RAC review and NIH approval for large-scale procedures; rather it specifies that large-scale experiments be approved by the IBC. She said the definition of large-scale might be revised. Dr. Gottesman agreed with Dr. Baltimore that the CDC Classification of Etiological Agents is not perfect, but she said the alternative in the December 4 proposal of "use

whatever you have and figure it out yourself" is not better. If RAC cannot find a better mechanism than the CDC classification, IBCs and PIs individually will not be able to make better decisions.

Ms. King said that the central issue is mandatory vs. voluntary guidelines. She said she was concerned with questions of process. She referred to Dr. Baltimore's statement that only a minority of scientists believe there may be some safety concerns with respect to recombinant DNA research. She said the public cannot ascertain whether that statement is accurate. The RAC did not cross-examine those who submitted written comments. Ms. King said RAC members should be aware of what she considers to be defects in process, and, therefore, err on the side of caution in deciding between the December 4 and December 7 proposals.

Dr. Nightingale praised the more extensive attempts to solicit comments on these proposals than had occurred in the past. As a result of this, the comments received were more varied than in the past. However, she felt it was only one small step in really assessing what the public feels. Referring back to Dr. Baltimore's statement, Dr. Nightingale said that disagreement does exist within the scientific community on whether there are unique risks of recombinant DNA research. She said that a major issue is voluntary vs. mandatory IBCs. She said that the December 7, 1981, proposal could be simplified and reorganized to make it easier to read and less cumbersome. She suggested that Section III-C could be eliminated; that the criteria for defining large-scale could be revised to emphasize inoculum size rather than volume; that Section IV could be simplified and reorganized; that the bureaucracy within IBCs could be greatly simplified; that the section dealing with whole or defective viruses could be simplified; that Sections III-B-2-a and Section III-B-2-b dealing with etiological agents could be combined; and that all work in nonpathogens could be performed at P1 containment. She viewed the December 7, 1981, proposal as a first, very positive step towards reducing complexity and restrictions.

Dr. Levine attempted to address the question of why recombinant DNA research is singled out for special consideration while other biomedical research, using inherently much more dangerous organisms, is not. He said the answer is in the historical context. Work with pathogens has had an extraordinary safety record for decades. The reason there was so much interest in control of recombinant DNA is that recombinant DNA technology became available in the 1970s, in an era of regulation. He cited procedures for research involving human subjects, which changed drastically in the early 1970s. He said he supports these constraints as they protect the public, as well as individual subjects, and they facilitate communication between the public and clinical investigators. He said being responsive to the public is very important and if a significant segment of the public is still concerned about recombinant DNA, this committee should be sensitive to that concern. He said that he would like to see something like the December 4, 1981, proposal ultimately adopted, but not immediately.

Dr. Ahmed said he wished to quote and highlight several points from the letter from the Public and Scientific Affairs Board of the American Society for Microbiology. He quoted from that letter that:

"Our concern is for the fact that only sparse information is available for other host-vectors. With less characterized systems, new combinations may result in organisms with potentially increased pathogenicity than either the donor or the recipient.

"We are not only concerned with the paucity of information but also with the lack of mechanisms for its dissemination. Many workers using modern genetic technology are not versed in pathogenic microbiology and cannot be assumed to have proper training or access to up-to-date information."

Dr. Martin said he believed as a scientist that recombinant DNA should not be singled out for special oversight. However, this position must be viewed within the historical context. He said that the state legislators and county supervisors with whom he had spoken are not primarily interested in the scientific basis for relaxation or elimination of the Guidelines, but rather in public opinion. RAC must be careful not to excite a public reaction that could result in greater bureaucratic and regulatory problems from local jurisdictions.

Dr. Saginor said he would like to propose an amendment to the December 7, 1981, proposal, should it pass, that a working group be formed to further refine, simplify, and reorganize that proposal, and that this group report to the RAC at a future meeting.

A discussion was held of the proper parliamentary procedure for the Committee to use to proceed. Mr. Thornton suggested that the Committee might vote now on Dr. Nightingale's motion to substitute the December 7 proposal for the December 4 proposal. This would result in the Committee choosing which "vehicle" it wished initially to adopt. Following this, RAC members could propose amendments to "perfect" the vehicle chosen, before the final vote on it.

Dr. Baltimore "called the question." By a vote of nineteen in favor, two opposed, and no abstentions, the RAC agreed to limit further debate and to vote on the motion to substitute the December 7, 1981, proposal for the December 4, 1981, proposal as the vehicle to be used for further amendments. Dr. Baltimore said that although, following this vote, any aspect of the winning proposal would be open for further amendments, he felt the vote should be viewed as a decision about whether "to go in the voluntary or mandatory direction." Dr. Nightingale reminded the RAC that her motion included the commitment to work towards future simplification of the Guidelines. By a vote of sixteen in favor, five opposed, and no abstentions, the RAC adopted the substitute motion, thus, choosing the Gottesman proposal as the vehicle to be placed before the Committee, open to further amendments.

Mr. Thornton recognized Dr. Susan Wright. Dr. Wright focused her comments on large-scale applications as she thought that while many other issues are being addressed, the RAC was not adequately addressing that issue. She said the primary focus of RAC has been on the hazards of research, not the hazards of industrial processes. She said that one cannot dismiss change of scale with regard to accidental release of recombinant organisms. She felt the data base on industrial hazards is very poor. She said she had heard some industrialists in other countries were considering using open fermentation tanks. If there is no oversight, companies will use whatever fermentation process they think is in their best interest. There are irresponsible companies willing to cut corners and take risks to try to gain a competitive advantage over responsible companies. Furthermore, there are no risk assessment experiments with organisms making insulin, interferon, etc. She said the committee is assuming that whatever product is being made will be harmless.

Dr. Wright said the RAC recommendation at the previous meeting to exempt from NIH review, certain large-scale experiments utilizing E. coli K-12, Saccharomyces cerevisiae and Bacillus subtilis host-vector systems was an error which produced a major gap in oversight. She urged the RAC to reconsider and re-evaluate its oversight over large-scale work.

Dr. Irving Johnson of Eli Lilly and Company said that industry has produced hundreds of gallons of the causative agents of polio, diphtheria, whooping cough, etc., with no great hazard to workers or to the environment, and in fact with great benefit to the population. Dr. Johnson said the only open vats he is aware of are in the beer brewing industry. Most industrial fermentations are generally highly contained to protect against contamination. Inocula are introduced into the growth tank through a rigid stainless steel structure. The connection does not leak and is steam sterilized.

Dr. Wright said she was not making a categorical statement about hazards, but rather about the data base. In her opinion, the data are extremely poor and incomplete, and assumptions that problems will be uncomplicated or easy to deal with are premature. These new technologies should remain under RAC review until a better data base develops.

Dr. Mason said that many industrial issues, though of concern, are beyond the scope of the RAC. Federal, state, and local authorities that make on-site inspections may wish to evaluate these issues, but RAC should not. Dr. Ahmed felt a distinction should be drawn between organism concentration and total amount in industrial processes.

Dr. Gottesman said that the December 7, 1981, proposal still requires that non-exempt large-scale procedures be reviewed by the local IBC before the project begins; P1-LS containment would still apply. It extends to all large-scale experiments the conditions approved by RAC at the previous meeting for certain large-scale experiments.

Dr. Berns questioned the language of Section I-B, Definition of Recombinant DNA Molecules, in the December 7, 1981, proposal. The relevant text of Section I-B reads as follows:

"Synthetic DNA segments likely to yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent) shall be considered as equivalent to their natural DNA counterpart. If the synthetic DNA segment is not expressed in vivo as a polynucleotide or polypeptide product, it is exempt from the Guidelines."

Dr. Gottesman pointed out that this is a reformulation of text which appears as Section III-E of the current (July 1, 1981) Guidelines. Dr. Berns suggested the real issue is whether the synthetic fragment would produce a biologically active product; he proposed to amend the language by adding the phrase "biologically active" before the word "polynucleotide" in the last sentence. Dr. Nightingale, who had proposed the motion being considered, and Dr. Fedoroff, the seconder of the motion, accepted the amendment.

Dr. Saginor then proposed an amendment which would explicitly state that a working group be appointed to review and attempt to simplify further the Guidelines and to report to the RAC at a future meeting. Dr. Nightingale, noting this intent was part of her original motion, accepted the amendment, as did Dr. Fedoroff.

Mr. Thornton called the question on Dr. Nightingale's motion as modified by amendments. By a vote of seventeen in favor, three opposed, and no abstentions, the RAC recommended adoption of the December 7, 1981, proposal with amendments. Mr. Thornton said a working group to refine the proposal would be designated at a later date, in accordance with the motion.

Dr. McGarrity asked the committee to state for the record that RAC sees no need for additional state and local ordinances governing recombinant DNA activities. Dr. Liberman, the biological safety officer at MIT, advised against adoption of Dr. McGarrity's statement as he viewed it as counterproductive. Based on his experience as a member of the Boston Biohazards Committee, he sees growing community interest in overseeing non-recombinant biohazards as recombinant systems are being handled.

Dr. Ahmed said he thought adoption of Dr. McGarrity's statement would be viewed as arrogance on the part of the RAC, saying "our views are gospel, and don't second guess us."

Mr. Mitchell said that he is in sympathy with the motion since he is concerned about fragmentation at the state and local level. However, knowing the independence of legislative bodies, it might not be well taken. He suggested that if the statement were reworded it might be more successful. Dr. McGarrity agreed and withdrew the proposal in order that revised text could be prepared for consideration later in the meeting.

Dr. Mason spoke against Dr. McGarrity's proposal, as it runs counter to usual regulatory practice, in which states and localities may regulate as long as their requirements are at least as stringent as Federal requirements. He added that RAC's recommendation would not be binding.

Dr. Holmes made a motion that there be added to the Guidelines a statement to the effect that:

"It is not clear that the biohazards associated with recombinant DNA are unique or different from biohazards associated with other work with pathogenic organisms; therefore, RAC encourages local Institutional Biosafety Committees to establish procedures for review of experiments not involving recombinant DNA, which, nonetheless, involve biohazards such as those addressed in Section III of the Guidelines."

Dr. Fedoroff seconded the motion. Dr. Landy suggested that a different statement be substituted for Dr. Holmes' proposed language to the effect that:

"The Recombinant DNA Advisory Committee wants to point out the absence of demonstrated risk or danger posed by recombinant DNA research. The continuance of the Guidelines for recombinant DNA research is made with full appreciation of the fact that other areas of research in which some risk has been demonstrated are without analogous guidelines."

Dr. Landy said such a statement would make clear to the public that RAC's recommendation to maintain guidelines is not based on demonstrated risk, but on potential risk.

Dr. Ahmed asked whether NIH has the authority to expand the purview of the IBCs as in Dr. Holmes' statement. Dr. Talbot replied that such a statement could be sent to the IBCs as a recommendation.

Dr. Goldstein said that he thought Dr. Landy's proposal could "stir up a hornet's nest," regenerating the situation of previous years with recombinant DNA. Ms. King said she could not support Dr. Landy's proposal as she questioned the phrase "absence of demonstrated risk." Dr. Berns moved to table Dr. Holmes' proposal. By a vote of seventeen in favor, three opposed, and no abstentions, the proposal was tabled.

Dr. Levine called the committee's attention to the report of the Working Group on Revision of the Guidelines entitled "Evaluation of the Risks Associated with Recombinant DNA Research" and particularly Part IV-A of the report, "Summary Analysis of Risks" (46 FR 59390). He said the conclusion is that most potential recombinant DNA risks envisaged in 1975 are now considered nonexistent. Ms. King said RAC should emphasize that available data cited in that report support and justify RAC's recommendation of the December 7, 1981, proposal. She suggested the RAC might formally reaffirm the "Summary Analysis of Risks." Dr. Martin suggested this text

might be used as a preamble to the introduction of the new Guidelines by the NIH Director. Dr. Holmes moved that the Director is requested to consider the "Summary Analysis of Risks" (46 FR 59390) as he determines a preamble to the revised Guidelines. Dr. Nightingale seconded the motion. She emphasized that her earlier motion for adoption of the December 7, 1981, proposal was based on the document "Evaluation of the Risks Associated with Recombinant DNA Research." She expected this document would be published as an integral part of the decision document. Dr. Mason hoped the document would note the different options considered by the RAC.

Ms. King suggested Dr. Holmes' motion be amended to call the attention of the NIH Director not just to the "Summary Analysis of Risks" (46 FR 59390) but also the motion which originally established the Working Group on Revision of the Guidelines, the Working Group's agenda, and its complete report.

Dr. Talbot asked if the motion might not be withdrawn, with the assurance that NIH staff would bring all of these items to the Director's attention without the necessity of a motion. Ms. King said she would prefer a specific motion since the Working Group report had not been formally endorsed by the RAC at the September 1981 meeting and since the RAC action today accepting the December 7, 1981, proposal is based on that report. Dr. Holmes reworded his motion to request the summary information discussed be included in the Director's preamble. Dr. Nightingale, who had seconded Dr. Holmes' earlier motion, also agreed.

Dr. Wright said that if there were to be a general statement on risks, then it should be made clear which industrial problems the RAC is not dealing with, so that no one thinks this is a global statement covering both research and industrial risks. Dr. Ahmed suggested that language be inserted indicating that the report does not address industrial scale-up. Dr. Berns noted that the NIH, on the advice of the RAC, had issued "Physical Containment Recommendations for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules;" RAC, however, is no longer evaluating mechanical details in individual large-scale applications. Dr. Landy opposed Dr. Ahmed's suggestion on the introduction of a specific statement on industrial considerations as it would dilute the general policy statement.

Dr. Pinon moved to table the motion; he preferred that ORDA bring these items to the attention of the Director, NIH, without the necessity of a formal motion. By a vote of ten in favor, eight opposed, and two abstentions, the motion to table carried.

After a brief recess, Dr. Mason moved to reconsider the action in order to provide the Director with a clear indication of RAC intent. He felt the previous vote revolved about procedural issues rather than intent. By a vote of ten in favor, four opposed, and three abstentions, the motion to reconsider was adopted.

Ms. King then moved that "the RAC specifically call to the Director's attention that the action taken on the December 7, 1981, proposal results from analysis and consideration of the report entitled 'Evaluation of the Risks Associated with Recombinant DNA Research' prepared by the Working Group on Revision of the Guidelines. The vote on the December 7, 1981, proposal implements the Working Group report."

By a vote of nineteen in favor, none opposed, and one abstention, the RAC adopted Ms. King's motion as a substitute for the previous motion. Mr. Thornton then ruled that unless there were objection (which there was not), the substitute motion is adopted by unanimous consent as the recommendation of the RAC.

Following an overnight recess, Mr. Thornton called the committee to order to consider language developed by Dr. McGarrity and Mr. Mitchell regarding local and state legislation. Mr. Mitchell moved acceptance of the following language:

"Whereas RAC has voted to recommend significant reductions in mandatory guidelines regarding recombinant DNA activity, and

"Whereas RAC in establishing said reduced guidelines did so based upon collective credible scientific knowledge and experience, and

"Whereas RAC believes it to be in the best interest of recombinant DNA activity to have a central arena for the dissemination of information and continuous review, and

"Whereas RAC believes the existence of uniform guidelines thereby establishes certainty and clarity in the scientific community, and

"Whereas RAC believes it would be detrimental to the advancement of recombinant DNA activity to have fragmentation of guidelines across the country,

"Therefore, be it resolved that RAC strongly recommends that local and state governments defer to the NIH Guidelines if enacting legislation governing recombinant DNA activity, unless it clearly establishes by credible scientific evidence that unique risk in fact exists in their particular jurisdiction."

Dr. McGarrity seconded the motion. He said the RAC action taken yesterday on the December 7, 1981, proposal would significantly relax the Guidelines. When considered in the context of possible additional local legislation, Mr. Mitchell's statement expressed RAC's judgement that the NIH Guidelines are the best possible approach at this time. It would be counterproductive for RAC to strip away bureaucracy and paperwork at the national level, only to have more bureaucracy and paperwork added at the state and local level.

Dr. Miller of the FDA strongly endorsed the sense of the motion. He said, almost without exception, the mosaic of local regulations has been more draconian, much less enlightened than the NIH Guidelines, and slower to evolve.

Dr. Martin suggested that the phrase "best interests of the public" be substituted for the phrase "best interest of recombinant DNA activity."  
Mr. Mitchell agreed.

Dr. Nightingale requested a clarification of the word "activity" in the motion. Mr. Mitchell replied that "activity" is an all inclusive term meant to cover research, development, production, etc.

Drs. Ahmed and Goldstein supported the sentiment expressed by the motion. However, Dr. Goldstein said he would vote against the language as he felt local communities would regard it as arrogant. Mr. Mitchell said he had chosen the verb "defer" to avoid the appearance of arrogance. The language urges that any actions be based on scientific grounds, and places the burden of proof upon advocates of local action. Dr. Friedman agreed.

Dr. Ahmed asked whether addition of the phrase "in as much as possible" would soften the language of the sentence:

". . . therefore, be it resolved that RAC strongly recommends that local and state governments defer to the NIH Guidelines . . ."

Mr. Thornton thought the verb "defer" alone was actually softer.

Dr. Mason said he could envisage situations in which local action might be necessary because of irresponsible action by a local academic or industrial group. He hoped RAC did not intend to say that local action should not be taken in such cases. Dr. Goldstein stated that communities realize that while universities are under sanctions, industry is not. Dr. Berns said that Mr. Mitchell's language specifies that when local entities legislate, they should defer to the NIH Guidelines in the scientific component of the legislation.

Dr. Mason said that many aspects of industrial scale-up are not covered by the Guidelines, yet the proposed language implies the existence of such guidance. He questioned whether RAC might amend the language to remove such implications. Dr. McGarrity suggested the phrase "DNA activity" be modified to "DNA research activity."

Dr. Ahmed said he supported the resolution but would prefer that a statement, delineating the scope of RAC activities, be appended to the language. If the committee could not formulate such a statement today, he hoped the Director's preamble to the acceptance of the December 7, 1981, proposal would state that neither RAC nor the NIH deals with mechanical aspects of industrial scale-up activities.

Dr. Pinon requested that the word "credible" be deleted from the phrase "credible scientific evidence"; he thought the term redundant. Dr. Saginor, however, disagreed as he felt "scientific" and "credible" are not synonymous to the public.

Dr. Gottesman saw the proposed language as intending to say to local legislators "we are listening to your concerns, we believe we are responding to them, and we hope you will continue to have faith in RAC." She warned, however, that the language might lead legislators who had not previously thought of legislation to consider it. Dr. Nightingale concurred. She thought acceptance of Mr. Mitchell's statement might be counterproductive. Instead she suggested that the Director's preamble to the revised Guidelines might state that these Guidelines are based on the best available information, and it is hoped they will be applied nationally. She preferred this procedure to a motion indicating RAC's concern over possible local legislation. Dr. Holmes agreed, expressing concern that the motion appeared arrogant and would be counter-productive. Dr. Berns called for the question.

By a vote of sixteen in favor, none opposed, and no abstentions, the RAC voted to stop debate and to vote on the motion proposed by Mr. Mitchell, as amended. By a vote of six in favor, nine opposed, and one abstention, the motion offered by Mr. Mitchell was defeated.

#### V. REQUEST TO CLONE SUBGENOMIC SEGMENTS OF FOOT AND MOUTH DISEASE VIRUS

Dr. Berns introduced the proposal (tabs 1058, 1059, 1062/1, 1063) of Molecular Genetics, Inc., to clone subgenomic segments of the Foot and Mouth Disease Virus (FMDV) in E. coli K-12. According to U.S. law, whole FMD virus cannot be studied in the U.S. except at the Plum Island Animal Disease Center (PIADC) of the U.S. Department of Agriculture (USDA). Dr. Berns said FMDV causes a disease with serious economic consequences; it is widespread globally but has been eradicated in the U.S.

Dr. Berns said Molecular Genetics, Inc., has cloned portions of the FMDV genome in Argentina. They have characterized the clones and have sent them to Plum Island for infectivity testing. Molecular Genetics, Inc., requests permission to remove these clones to their laboratories in Minnesota. The clones represent, in aggregate, less than sixty-five percent of the FMDV genome. Dr. Berns recalled that Genentech, Inc., in collaboration with USDA had approached the NIH with a similar proposal. That project was approved by RAC and subsequently by the NIH. Genentech, Inc., had received permission to remove clones representing in aggregate seventy-five percent of the FMDV genome from Plum Island to Genentech, Inc., laboratories in California. Dr. Berns recommended approval of the Molecular Genetics, Inc., proposal.

Dr. Tolin said the USDA is waiting for RAC review of the project before testing the infectivity of the clones. She said she had reviewed the documents submitted by Molecular Genetics, Inc., and found them to be in order.

Dr. Maas asked how the FMDV strains cloned by Molecular Genetics, Inc., differed from the strains cloned by Genentech, Inc. Dr. Pilacinski said the FMDV strains cloned by Molecular Genetics, Inc., are indigenous to Argentina and Latin America.

Dr. Ahmed asked why Molecular Genetics, Inc., had requested P1 containment conditions for the work in Minnesota. Dr. Berns said that P1 conditions had been previously approved for the subgenomic FMDV clone work conducted by Genentech, Inc., in California. Dr. Goldstein asked what scale experiments Molecular Genetics anticipated. Dr. Collett said the work would be laboratory scale.

Dr. Berns moved approval of the request. Dr. McKinney seconded the motion. Dr. Talbot clarified the language of the motion: the motion would provide for review of USDA infectivity data by the FMDV Working Group of the RAC before NIH permission would be granted. This procedure had been followed in approving the Genentech, Inc., request. Dr. Goldstein inquired about the composition of this working group. Dr. Gartland said Drs. Baltimore, Berns, and Tolin currently compose the group, and additional members will be named. Dr. Goldstein said he wished that the decision of the working group be sent to the RAC. Dr. Berns agreed.

Mr. Thornton called the vote. By a vote of sixteen in favor, none opposed, and four abstentions, the motion was approved.

#### VI. PROPOSED INCLUSION OF YERSINIA ENTEROCOLITICA ON SUBLIST A OF APPENDIX A

Dr. Fedoroff said that tabs 1052 and 1056C/6 present a request from Dr. Guy Cornelis of the Universite Catholique de Louvain, Brussels, Belgium. Dr. Cornelis requested that Yersinia enterocolitica be exempted from the Guidelines under Section I-E-4 and added to Sublist A, Appendix A. Dr. Fedoroff said Y. enterocolitica exchanges genetic information with E. coli with a frequency of transfer roughly three orders of magnitude lower than seen in exchange between E. coli and E. coli. Under certain conditions, that frequency can be enhanced. Mutants which have higher exchange frequencies can also be selected.

Dr. Fedoroff asked Dr. Levine to comment on Yersinia enterocolitica. Dr. Levine said that some strains of Yersinia enterocolitica cause disease in man. The disease producing serotypes are invasive, with some producing a heat stable enterotoxin whose mechanism of action is identical to that of heat stable E. coli enterotoxin. In school age children, Y. enterocolitica is a major cause of mesenteric adenitis which leads to a pseudo-appendicitis type syndrome. In older individuals one sees hypersensitivity reactions, including erythema nodosum; in individuals of the HIAV27 allotype, chronic arthritis may develop following Yersinia infection.

Dr. Levine said the data demonstrate genetic exchange with E. coli in the test tube, and this exchange probably occurs in nature. On that basis, he supported the proposal. Dr. Fedoroff moved approval of the proposal. Dr. Levine seconded the motion. By a vote of eighteen in favor, none opposed, and one abstention, the RAC approved the motion.

#### VII. PROPOSED PSEUDOMONAS PUTIDA HOST-VECTOR SYSTEM

Dr. Maas introduced the proposal (tabs 1053, 1056C/5) of Dr. Michael Bagdasarian of the Max-Planck Institut fur Molekulare Genetik, Berlin, West Germany. Dr. Bagdasarian requested HVI certification of a host-vector system based on Pseudomonas putida strain KT2440 and plasmid cloning vectors pKT262, pKT263 and pKT264.

Dr. Maas questioned whether it is appropriate for RAC to consider a proposal which originated with an investigator outside the U.S. Mr. Thornton replied that if the system could be widely applied in research, RAC might appropriately evaluate it for certification. Drs. Maas, Fedoroff, and McKinney said a P. putida host-vector system could be widely used.

Dr. Maas said the information provided supports the investigator's request for HVI certification, and so moved. Dr. Berns seconded the motion, adding that a P. putida HVI system would be very useful as genes which are not expressed in E. coli host-vector systems may be expressed in P. putida systems. Mr. Thornton called the motion. By a vote of eighteen in favor, none opposed, and one abstention, the RAC approved the motion.

#### VIII. PROPOSALS INVOLVING EK2 HOST-VECTOR SYSTEMS

##### A. Proposed EK2 Host-Vector Systems

Dr. Friedman introduced the proposal (tabs 1054, 1056C/3) of Dr. Roy Curtiss of the University of Alabama, Birmingham, Alabama. Dr. Curtiss requested EK2 certification of six different E. coli K-12 strains in conjunction with various virulent and temperate bacteriophage lambda, plasmid, and cosmid vectors. Dr. Curtiss also requested that all previously approved EK2 vectors be approved as vector components of the proposed EK2 host-vector systems.

Dr. Friedman said the proposed EK2 host-vector systems are:

- (1) E. coli K-12  $\chi$ 2447, and its suppressor-free sib  $\chi$ 2281, for use with virulent bacteriophage lambda vectors including specifically, but not limited to, Charon 4A.

- (2) E. coli K-12  $\chi$ 1984, and its suppressor-free sib  $\chi$ 2705, for use in conjunction with: virulent lambda vectors including but not limited to Charon 4A; temperate bacteriophage lambda vectors  $\lambda$ YEQS cI857 and  $\lambda$ ZEQS cI857; plasmid cloning vector pBR322; and the cosmid cloning vectors pJC75-37, pJC75-58, pJC76, pJC77, and pHC79.
- (3) E. coli  $\chi$ 2001 and its suppressor-free sib  $\chi$ 2363, for use in conjunction with all of the vectors enumerated in part two for  $\chi$ 1984 and  $\chi$ 2705 plus the cosmid vector pJC78.

Dr. Friedman said that an ad hoc working group held a telephone conference call on January 21, 1982, to discuss this request; that discussion is summarized in Attachment II. He then suggested that RAC evaluate part one of the proposal separately. He said the ad hoc working group agreed that the systems described in part one,  $\chi$ 2447 and  $\chi$ 2281 with the virulent bacteriophage lambda vectors, meet EK2 certification criteria. The major safety feature of these systems resides in the vectors rather than in the host; nonetheless, the hosts meet the EK2 requirements specified in the Guidelines.

Dr. Maas requested an explanation of how the suppressor-free sibs would be used. Dr. Gottesman said the suppressor-free sibs would be used to test the virus for reversion; they would not be used for propagating cloned material.

Dr. Talbot suggested that a motion be offered on the first part of the proposal. Dr. Friedman moved that strains  $\chi$ 2447 and  $\chi$ 2281 in part one of the proposal be approved for use with those lambda vectors certified for use in DP50 on the condition that the suppressor-free strain not be used as a propagation host.

Mr. Thornton called the vote. By a vote of eleven in favor, none opposed, and two abstentions, the committee approved the motion.

Dr. Friedman suggested that parts two and three of the proposal be discussed together as both have the same problems. In addition to requesting permission to utilize virulent lambda phage as vectors, Dr. Curtiss requests, in parts two and three, certification for lysogenizing lambda phage and for plasmid and cosmid vectors.

Dr. Friedman said Dr. Curtiss presented no data, as required for EK2 certification, on the lysogenizing phages or for the cosmid vectors. In order to approve plasmid vectors, data from triparental matings must be evaluated, however, Dr. Curtiss supplied no data pertinent to triparental matings in strains  $\chi$ 1984 and  $\chi$ 2705 nor for  $\chi$ 2001 and  $\chi$ 2363.

Dr. Gottesman explained the rationale behind the EK2 approval procedure. There are two considerations: (1) whether the host could establish and spread in the environment, and (2) whether the organism could disseminate recombinant DNA to secondary hosts. She explained

that virulent lambda vectors containing certain mutations will not persist in the environment; the survivability of the host in the environment is then a less important consideration. For this reason virulent lambda vectors can be certified without too much data on host survivability being evaluated.

With the plasmid, cosmid and lambda lysogen vectors, whether the host establishes in the environment is a more important consideration. The data Dr. Curtiss supplied on survival for the proposed host strains are less than were evaluated for the EK2 approved host  $\chi$ 1776. Furthermore, Dr. Curtiss does not provide enough data on the proposed cosmid and lambda lysogen vectors. These portions of the proposal should be rejected as supporting data are lacking. Dr. Gottesman suggested that for certain plasmids, the available data may be adequate to warrant approval. The systems using virulent lambda vectors should perhaps be certified.

Dr. Friedman said the ad hoc working group did not recommend approval of those sections of the proposal dealing with lysogenizing phage vectors, plasmid, and cosmid vectors. He recommended approval of strains  $\chi$ 1984,  $\chi$ 2705,  $\chi$ 2001, and  $\chi$ 2363 when virulent lambda phages are used as vectors.

Dr. Friedman asked if the RAC had specified criteria for certification of lysogenizing lambda. Dr. Gottesman replied that they have not; no previous submissions dealing with lysogenizing phages were received. She thought the testing criteria for cosmids might be applicable to lysogenizing phages.

Dr. Levine expressed his concern over the testing criteria specified for EK2 certification. He noted that at the time the EK2 criteria were designed, no data on  $\chi$ 1776 survivability in man were available. When those data became available, it was discovered that  $\chi$ 1776, containing pBR322, survived longer in man than  $\chi$ 1776 without pBR322. Data generated from the mouse system did not predict this phenomenon. He suggested that another level of testing be added to the EK2 criteria: feeding experiments in man should be performed, as these yield the most pertinent data.

Dr. Talbot suggested that any redefinition of EK2 criteria should be considered by the EK2 working group which could report at the next RAC meeting. At this meeting, RAC should use current criteria to evaluate Dr. Curtiss' proposal. Mr. Thornton concurred. Dr. Levine suggested that a motion to defer consideration of parts two and three of Dr. Curtiss' proposal might be in order.

Dr. Ahmed asked if the EK2 certification criteria would be changed by the recommendation on revising the Guidelines made earlier in the meeting (Item IV above). Drs. Talbot and Gottesman replied that it would not. Dr. Martin asked if  $\chi$ 1776 would fall under a grandfather

clause should EK2 certification criteria be changed. He wondered whether  $\chi$ 1776 would qualify as an EK2 host under the new criteria. Dr. Levine replied that those questions would have to be considered by the EK2 working group. One possibility would be to accept, as a maximum permissible level, the survival values of  $\chi$ 1776 in the human gut. Alternatively, the working group may have to deal with the possibility that  $\chi$ 1776 is not as debilitated in the human gut as anticipated. Dr. Liberman pointed out that other systems have been decertified, and urged that the EK2 working group reevaluate  $\chi$ 1776's status as an EK2 certified vector.

Dr. Gottesman suggested parts two and three of Dr. Curtiss' request should be deferred as: (1) the information provided is not adequate to evaluate the host-vector systems vis-a-vis the EK2 criteria, and (2) if the EK2 criteria is reconsidered, reconsideration would have important implications for Dr. Curtiss' proposal.

Dr. Friedman moved that the four hosts ( $\chi$ 1984,  $\chi$ 2705,  $\chi$ 2001, and  $\chi$ 2363) be accepted for use with the virulent lambda vectors on condition that the suppressor-free strains not be used as propagation hosts. Consideration of cosmid, plasmid and lysogenic lambda vectors is deferred until more information is obtained. Dr. Maas seconded the motion.

Mr. Thornton called the motion. By a vote of eleven in favor, none opposed, and four abstentions, the RAC approved the motion.

Dr. Levine said that

"Whereas EK2 systems imply and are meant to result in a high degree of containment, and

"Whereas bacterial hosts in such systems are meant to be highly defective in their ability to survive in the environment, as well as in mammalian intestine, and

"Whereas the guidelines for EK2 criteria were designed before much credible scientific data on these points were available, and

"Whereas data have recently come to light from human feeding experiments with EK2 hosts with and without plasmid pBR322 that demonstrated increased persistence of the host containing plasmid pBR322,"

he would move that the certification criteria for EK2 host-vector systems be reconsidered by the EK2 working group specifically to consider making human feeding studies which yield the most relevant data, one of the criteria.

Dr. Ahmed asked if the working group would report to RAC. Mr. Thornton said it would. It was stated that while many RAC members would agree to have the certification criteria reconsidered by the EK2 working

group, not all would necessarily agree with Dr. Levine's "whereas" preamble. Mr. Thornton noted the "whereas" statement was merely prefatory, and not formally part of the motion. By a vote of fourteen in favor, none opposed, and four abstentions, the RAC accepted the motion.

Dr. Mason asked if the Working Group on Revision of the Guidelines would consider this motion. Dr. Talbot replied they would.

B. Proposed Use of EK2 Host-Vector Systems for Cloning DNA from Class 3 and 4 Etiologic Agents

Dr. Friedman then began discussion of a second proposal (tabs 1054, 1056C/4) from Dr. Roy Curtiss of the University of Alabama. Dr. Curtiss requested permission to use all certified EK2 host-vector systems to clone DNA fragments from Class 3 and Class 4 etiologic agents under P2 containment conditions. P1 containment could be employed if the recombinant clones are shown not to express a virulence determinant that has toxic potential.

As an alternative, if this general proposal were not accepted by RAC, Dr. Curtiss requested permission to clone DNA from Yersinia pestis and Mycobacterium leprae into EK2 host-vector systems under P2 containment conditions. P1 conditions could be employed if virulence determinants are not expressed by the recombinant clones.

Dr. Friedman asked Dr. Gottesman how Dr. Curtiss' proposal dealing with Class 3 agents would be treated under the proposed revision of the Guidelines recommended earlier by RAC (Item IV above). Dr. Gottesman replied that in the proposed revised Guidelines experiments, in which DNA from a Class 3 etiologic agent is cloned in a nonpathogenic prokaryote, could be performed under P2 containment conditions. Under the current Guidelines, DNA from Class 3 agents may be cloned in EK1 hosts under P3 containment conditions. She suggested it would be consistent with the current Guidelines to permit the investigator to lower physical containment to P2 if biological containment is raised to EK2.

Dr. Friedman moved that DNA from Class 3 agents may be cloned in EK2 host-vector systems under P2 containment conditions; Class 4 agents, however, should be dealt with on a case-by-case basis. Dr. Maas seconded the motion. By a vote of thirteen in favor, none opposed, and two abstentions, the RAC adopted the motion.

IX. REQUEST TO USE BACILLUS MEGATERIUM IN RECOMBINANT DNA EXPERIMENTS

Dr. Holmes introduced the request (tabs 1051, 1056C/2) of Dr. Patricia Vary of Northern Illinois University for permission to introduce recombinant DNA derived from Staphylococcus aureus, E. coli, and Bacillus subtilis into Bacillus megaterium under P1 conditions. In her letter of November 24, 1981, she also requested that B. megaterium be classified as

a genetic exchanger with B. subtilis under Section I-E-4. Dr. Holmes said the evidence supporting the request that B. megaterium be added to Appendix A, Sublist B, is weak. Dr. Gartland said that Dr. Vary had, after consulting with ORDA, withdrawn her request that B. megaterium be added to Appendix A. That part of her request was, therefore, not published in the Federal Register.

Dr. Holmes said that an issue in the Federal Register request is that the plasmids to be used are not specified. Dr. Holmes said B. megaterium is not an important pathogen in either human or veterinary medicine. It will, rarely, cause infections in compromised or immunosuppressed patients.

Dr. Berns asked if any problems could be visualized which would argue for setting containment any higher than P1. Dr. Berns said B. megaterium is only an opportunistic pathogen. Dr. Holmes pointed out that Dr. Vary could be introducing antibiotic resistance genes into B. megaterium. Dr. Levine pointed out that B. megaterium forms spores; spores are better contained under P2 conditions.

Dr. Holmes moved that the request to transfer recombinant plasmids from E. coli, B. subtilis, and S. aureus into B. megaterium be approved under P2 containment conditions. Containment is set at P2 as Dr. Vary has not specified the experiments she wishes to perform nor the plasmids to be used; however, she is encouraged to apply to her local IBC with specifics if she wishes containment lowered to P1, the local IBC is authorized to lower containment to P1 for specific experiments. Dr. Levine seconded the motion. By a vote of thirteen in favor, none opposed, and one abstention, the RAC approved the motion.

#### X. REQUEST TO CLONE PLANT DNA IN THE CYANOBACTERIUM ANACYSTIS NIDULANS

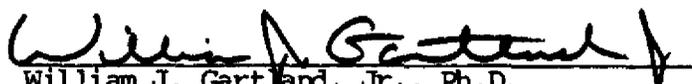
Dr. Scandalios introduced tabs 1055 and 1062/2, a request from Dr. Lawrence Bogorad of Harvard University for permission to initiate, at P1 containment, a program involving the cloning, in the cyanobacterium Anacystis nidulans (strain R2), of DNA from chloroplasts of various plants (initially primarily from Zea mays). Dr. Bogorad would employ the plasmid vector pUC104, a construct of the cyanobacterial plasmid pUC1 and the E. coli vector pACYC184. Dr. Scandalios said he had consulted Dr. Winston Brill by telephone on this proposal. Dr. Scandalios said neither he nor Dr. Brill could envisage any potential problems, so he would recommend that the experiments be permitted at the P1 containment level. He so moved. Dr. Levine seconded the motion. By a vote of fourteen in favor, none opposed, and no abstentions, the RAC approved the motion.

XI. FUTURE MEETING DATES

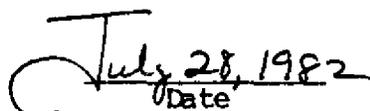
Mr. Thornton noted that the originally scheduled RAC meeting date of April 23-24, 1982, was probably earlier than necessary. Dr. Talbot suggested that the later part of June was most suitable and that ORDA would contact RAC members by telephone to determine the best possible date. Mr. Thornton thanked the members of the committee for their participation. He then adjourned the meeting at 11:22 a.m., February 9, 1982.

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.

  
 Date

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

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RECOMBINANT DNA ADVISORY COMMITTEE

AD HOC CONSULTANTS

February 8-9, 1982

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Summary of Conference Call on Proposed EK2 Host-Vector Systems  
Submitted by Dr. Roy Curtiss dated September 25, 1981

January 21, 1982

Drs. Campbell, Clewell, Friedman, Goldstein, Gottesman, Levine, Maas, and Gartland met by conference call on January 21, 1982, to discuss the submission of Dr. Roy Curtiss dated September 25, 1981.

Request 1 deals with use of  $su^+$  and  $su^-$  derivatives of DP50 with previously approved virulent lambda vectors. It was noted that most of the containment in these systems is provided by the lambda vector and that testing data have been provided for Charon 4A. Although the testing data are for a period of 8 hours rather than 24 hours, it was felt that the strains look at least as good as DP50. With regard to survival in rats following oral administration (Table 5, page 166), it was noted that only in vitro data are required for testing at the EK2 level. It was the consensus of the consultants that the strains in part 1 of the request be approved for use with those lambda vectors certified for use in DP50 on the condition that the  $su^-$  strain not be used as a propagation host.

The participants then reviewed parts 2 and 3 of the request. It was noted that these proposals include requests for use of virulent and temperate lambda vectors, as well as cosmids. It was agreed that with regard to the temperate phages and cosmids, additional data are needed on how the phages are constructed, how they behave in the host, how they persist, etc. The participants requested the information provided by Dr. Pierre Tiollais on the construction and properties of the temperate lambda vectors, and information provided by Dr. John Collins on the construction and properties of the cosmid vectors. They also requested a copy of "Section 4" of a report referred to by Dr. Curtiss and information on certification of cosmid vectors (These documents were mailed to the participants on January 26). Dr. Levine suggested that testing in humans should be done. Again it was pointed out that EK2 certification has never required in vivo testing.

The group was divided on how to handle parts 2 and 3 of the submission. Four participants (Drs. Campbell, Clewell, Friedman, and Maas) recommended that the strains in parts 2 and 3 be approved for use with plasmids certified for use in 1776 and for use with virulent phages on the condition that  $su^-$  strains not be used as propagation hosts. Dr. Gottesman said that she would prefer to vote only on the phage vectors. Dr. Goldstein abstained. Dr. Levine abstained on the basis that he does not agree with the criteria for EK2 systems. It was agreed that further consideration is needed on the request for use of temperate phages and cosmids with these hosts.

These recommendations will be transmitted to the RAC at its meeting on February 8-9, 1982.

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