

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

RECOMBINANT DNA ADVISORY COMMITTEE

MINUTES OF MEETING¹

MARCH 6-7, 1980

The Recombinant DNA Advisory Committee (RAC) was convened for its eighteenth meeting at 9 a.m. on March 6, 1980, in Wilson Hall, Building 1, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland. Dr. Jane K. Setlow, (Chairman) Biologist, Brookhaven National Laboratory, presided. In accordance with Public Law 92-463 the meeting was open to the public, except for the review of proposals involving proprietary information as the last item of business on March 7, 1980.

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

Dr. Abdul Karim Ahmed; Dr. David Baltimore; Dr. Kenneth Berns, Dr. Winston Brill; Dr. Francis Broadbent; Dr. Allan Campbell; Mrs. Zelma Cason; Dr. Richard Goldstein; Dr. Susan Gottesman; Dr. Jean Harris; Ms. Patricia King; Dr. Sheldon Krinsky; Dr. Werner Maas; Dr. James Mason; Dr. Elena Nightingale; Dr. David Parkinson; Dr. Samuel Proctor; Mr. Ray Thornton; Dr. LeRoy Walters; Dr. Luther Williams; Dr. Frank Young; Dr. Milton Zaitlin; 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. Robert W. McKinney, Enviro Control, Inc., Rockville, Maryland

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

Dr. Walter R. Dowdle, Center for Disease Control; Dr. Timothy J. Henry, Food and Drug Administration; Dr. Herman Lewis, National Science Foundation; Dr. David Logan, United States Department of Labor; Mr. Melvin Myers, National Institute for Occupational Safety and Health; Dr. Jane Schultz, Veterans Administration; Dr. Sue Tolin, United States Department of Agriculture; and Dr. William J. Walsh, III, Department of State.

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.

Other National Institutes of Health staff present were:

Dr. Edward Applebaum, NCI; Dr. Stanley Barban, NIAID; Dr. W. Emmett Barkley, ORS; Mrs. Betty Butler, NIAID; Dr. Irving Delappe, NIAID; Dr. John Irwin, ORS; Dr. Micah I. Krichevsky, NIDR; Dr. Richard Krause, NIAID; Mr. Charles Leasure, NIAID; Dr. Malcolm Martin, NIAID; Dr. Elizabeth Milewski, NIAID; Dr. Stanley Nagle, NIAID; Dr. John Nutter, NIAID; Dr. Joseph Perpich, OD; Mr. Richard Riseberg, OGC; Dr. Wallace Rowe, NIAID; Dr. Bernard Talbot, OD; and Dr. Rudolf Wanner, ORS.

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

Dr. Harvey C. Aaron, Occupational Safety and Health Administration; Dr. Queta Bond, National Academy of Sciences; Dr. K. C. Bora, Health & Welfare, Canada; Dr. Peter G. Bostock, New Brunswick Scientific Co., Inc.; Dr. J. Paul Burnett, Eli Lilly & Company; Dr. William Can, Jack Raymond & Company; Mr. Jeffrey Christy, Blue Sheet; Dr. Howard Eddy, Science Council of Canada; Dr. Russel K. Enns, Alpha Therapeutic Corp.; Dr. Stanley Falkow, University of Washington; Dr. Mark Finkelstein, Schering Corporation; Dr. Zsolt Harsanyi, Office of Technology Assessment; Ms. Linda Haverfield, Friends of the Earth; Dr. Paul Hung, Abbott Research Laboratories; Dr. Evelyn Hurlburt, Johns Hopkins School of Hygiene; Dr. I. S. Johnson, Eli Lilly & Company; Dr. Attila I. Kadar, Food and Drug Administration; Dr. Geoffrey Karny, Office of Technology Assessment; Dr. Charles C. Kimble, Food and Drug Administration; Dr. David W. Krogmann, U. S. Department of Agriculture; Dr. Paul Leibowitz, Schering Corporation; Dr. M. A. Levine, Environmental Protection Agency; Dr. F. R. Marks, University of California, San Francisco; Ms. Carmine Masucci, New Brunswick Scientific Co., Inc.; Mr. Bing Miller, New Brunswick Scientific Co., Inc.; Ms. Julie Miller, Science News; Dr. Henry I. Miller, Food and Drug Administration; Mr. J. S. Narrim, Virginia, Office of Governor; Dr. DeLill Nasser, National Science Foundation; Mr. Seth Pauker, National Institute for Occupational Safety and Health; Dr. James D. Punch, UpJohn Co.; Dr. Anthony Robbins, National Institute for Occupational Safety and Health; Dr. Michael Ross, Genentech, Inc.; Dr. Brian Sheehan, Genentech, Inc.; Dr. Richard Silver, Food and Drug Administration; Dr. J. R. Swarz, Staff, United States Senate; Dr. Vicki Weisfeld, Institute of Medicine; Dr. Susan Wright, University of Michigan; and Dr. W. P. Young, Eli Lilly & Company.

I. CALL TO ORDER AND OPENING REMARKS

Dr. Jane Setlow, chairperson, called the meeting to order at 9:00 a.m., March 6, 1980. Dr. Setlow introduced Dr. Kenneth Berns, a new Recombinant DNA Advisory Committee (RAC) member.

Dr. Setlow then called the attention of the RAC to two items, tab 847 and tab 848 which had been included in the material sent to the RAC. She noted that tab 847 is a report of the frequency in nature of Escherichia coli strains that can serve as recipients and/or hosts for genetic information carried by various Escherichia coli recombinant DNA host-vector systems. She said that tab 848 deals with the production of a choriogona-dotropin-like factor by a microorganism tentatively named Progenitor cryptocides.

II. MINUTES OF DECEMBER 6-7, 1979 MEETING

The RAC reviewed the minutes of the previous meeting (tab 863). Dr. Walters said he believed there were two substantive errors in the minutes: on page 27, line 32, a semicolon should follow the word "experimental;" on page 31, line 15 should read "host-vector systems are excepted from...."

Dr. Berns noted that on page 15, line 7, he was credited with making a motion concerning an RAC major action. He said he believed Dr. Baltimore actually made this motion.

Dr. Goldstein requested a cross indexing in the discussion of the Foot and Mouth Disease (FMD) proposal beginning on page 4, of the paragraph on page 23 which states that Dr. Krinsky submitted to the RAC material pertinent to the FMD discussion. In addition, he requested that an index of the material supplied by Dr. Krinsky be appended to the minutes.

Mr. Thornton moved approval of the minutes. The minutes, with the changes incorporated, were unanimously approved, with the exception of Dr. Parkinson who abstained.

III. REPORT OF MEETING OF FEDERAL INTERAGENCY ADVISORY COMMITTEE ON RECOMBINANT DNA RESEARCH

Dr. Joseph Perpich, Executive Secretary of the Federal Interagency Advisory Committee (FIC) reported on the FIC meeting (tab 817, 856) of February 27, 1980. He noted that Dr. Maxine Singer, NCI, NIH, had presented a summary of scientific advances in the area of recombinant DNA technology, Dr. William Gartland, ORDA, NIH, had reviewed the recent revisions in

the Guidelines, and Dr. Richard Krause, NIAID, NIH, had reported on the NIH recombinant DNA risk assessment program. Dr. Morris Levin had reported on the risk assessment program of the Environmental Protection Agency (EPA). Dr. Bernard Talbot, OD, NIH, had summarized the work of the RAC in reviewing industrial applications, and Dr. Barkley, ORS, NIH, had reviewed the proposed supplement for large-scale physical containment practices. Dr. Burke Zimmerman, OD, NIH, had briefly reviewed Senator Stevenson's bill, S. 2234.

Dr. Perpich said the FIC met primarily to be briefed on RAC activities and to consider what actions, if any, were necessary regarding the industrial application of recombinant DNA technology. Dr. Perpich reported that Dr. Bingham, Assistant Secretary for Occupational Safety and Health, Department of Labor, summarized the interest and involvement of the Occupational Safety and Health Administration (OSHA), and that Dr. Robbins, Director of the National Institute for Occupational Safety and Health (NIOSH), seconded her concerns. In response to these concerns, the Interagency Committee recommended that a subcommittee be created to examine occupational health issues attendant upon industrial application of recombinant DNA techniques. Dr. Perpich said this subcommittee, the Industrial Practices Subcommittee, will be chaired by Dr. Gilbert Omenn of the Office of Science and Technology Policy (OSTP), and will include representatives of the U. S. Department of Agriculture (USDA), the Department of Commerce (DOC), the National Institute for Occupational Safety and Health (NIOSH), the Center for Disease Control (CDC), the Food and Drug Administration (FDA), the National Institutes of Health (NIH), the Occupational Safety and Health Administration (OSHA), the National Science Foundation (NSF), and the Office of Science and Technology Policy (OSTP). Dr. Perpich said the minutes of the FIC meeting would be distributed to the RAC.

Dr. Parkinson stated his belief that OSHA has the authority to regulate the industrial application of recombinant DNA technology and questioned the need for another committee. Dr. Robbins of NIOSH said that OSHA is responsible for regulating workplaces employing recombinant technology as for workplaces using any other technology. He stated that presently there is no clear guidance as to what standards should be used to enforce the OSHA mandate. He said the absence of standards in terms of work practices, engineering, etc, does not eliminate OSHA's responsibility to protect the workplace and that OSHA and NIOSH have begun to examine this area. He added that NIOSH and OSHA do not want to wait a lifetime of worker exposure before examining potential hazards and instituting regulations. He said that if principles of protection are developed early in the industrial application of these techniques, implementation costs to industry would be substantially less than in a "retrofit" situation. Dr. Baltimore stated that regulation of industrial recombinant DNA practices should be part of the general question of regulating the industrial application of microorganisms. Dr. Robbins agreed.

Dr. Parkinson said there are aspects of medical surveillance which should be observed in any workplace where microbiological techniques are employed. Dr. Krimsky asked to what extent OSHA has been involved in the surveillance of this type of workplace. Dr. Parkinson responded that OSHA has not been involved to any great extent. Dr. Robbins pointed out that the notion of developing medical surveillance as part of workplace standards is relatively new.

Dr. Parkinson moved that the RAC request that OSHA begin the formal process of promulgating regulations addressing potential hazards of microbiological techniques in industry, including the innovative use of recombinant DNA techniques. Dr. Ahmed suggested broader language be used to permit greater flexibility. Dr. Parkinson accepted Dr. Ahmed's suggestion.

Mr. Thornton expressed concern over whether the RAC can request OSHA to initiate a particular course of action. He suggested that a request to the Director, NIH would be more appropriate. Dr. Parkinson agreed, and amended his motion to read that the RAC recommends that the Director, NIH, request OSHA to begin the process of initiating possible regulations in the area of the industrial use of microbiological techniques.

Noting that the RAC members had been communicating their concerns directly to the NIOSH and OSHA representatives present at the meeting, Dr. Baltimore stated that he saw no need for the motion and moved to table it. The motion to table was approved by a vote of fourteen in favor, five opposed, and one abstention.

IV. PROCEEDINGS OF THE NIAID WORKING GROUP ON RISK ASSESSMENT

Dr. Krause said that concern had been voiced that, under exceptional circumstances, Escherichia coli K-12 expressing eukaryotic proteins might produce or induce a toxic result. He said two individuals had been selected to assist NIAID in developing a risk assessment workshop to evaluate this concern: Dr. Louis Sherwood, Physician in Chief and Chairman, Department of Medicine, Michael Reese Hospital, Chicago; and Dr. Philip Paterson, Chairman and Professor, Department of Microbiology and Immunology, Northwestern University. Dr. Krause said that the conference would be held in Pasadena, California, on April 11 and 12, 1980. A general session outlining risk assessment in Escherichia coli K-12 will be followed by concurrent sessions dealing with (1) the hypothetical direct effects of active polypeptides and hormones chaired by Dr. Sherwood, and (2) possible autoimmune responses chaired by Dr. Paterson. Dr. Krause added that the meeting had been publicized and, thus far, a total of 55 people have registered to attend. These include three of the five members of the RAC Risk Assessment Subcommittee, seven non-voting members of the RAC, three members of the Federal Interagency Advisory Committee on Recombinant DNA Research, and representatives of three European nations.

Mr. Thornton requested that the RAC Risk Assessment Subcommittee consider whether other areas of concern exist; e.g., should risk assessment be performed with other host-vector systems. Dr. Zaitlin asked that the Risk Assessment Subcommittee consider the desirability of initiating risk assessment experiments in the plant pathology area.

Dr. Krause then introduced Dr. Stanley Falkow, from the University of Washington, who reported (tab 819, 858) on the unanimous recommendation of an NIAID Risk Assessment Working Group, which met on August 30, 1979, that NIAID not implement risk assessment protocols I and II developed from the proceedings of a Workshop held at Falmouth, Massachusetts, June 20-21, 1977. Protocol I, "Colonization and Transmission of Plasmids by Escherichia coli K-12 in the Gastrointestinal Tract of Humans," and protocol II, "Tests for Transmissibility of Plasmids of Escherichia coli K-12 and xl776 in Germ-Free Mice" had been developed to evaluate the probability that recombinant DNA, carried by an Escherichia coli K-12 host-vector system, might be transferred to other members of the flora. Dr. Falkow said the Risk Assessment Working Group, after evaluating the data generated by feeding Escherichia coli K-12 to over 60 different people, felt that these protocols would produce negative data. The Working Group, therefore, suggested that a "worst case" type of experiment be substituted for these two protocols. The alternative protocol would utilize instead of strain K-12 a "wild type" Escherichia coli strain, HS, which is known to be nonpathogenic and to colonize the majority of normal individuals who ingest it. The Working Group recommended that the cloning vehicle pBR325, coding for chloramphenicol resistance, be transformed into the HS strain, and that a wild type mobilizable plasmid such as Cole1 carrying chloramphenicol resistance be used as a control. The group felt that this protocol would generate basic scientific information, i.e., mobilization frequencies in the gut, as well as provide risk assessment data for recombinant DNA.

Dr. Falkow said that the NIAID Risk Assessment Working Group had in addition discussed the question of whether Escherichia coli K-12 can be converted into a pathogen. He said that no members of the Working Group, who collectively possess over 20 years of experience in attempting to make K-12 pathogenic, had succeeded in creating a pathogenic K-12. He described some of the attempts.

Dr. Krimsky asked Dr. Falkow why K-12 is difficult to convert into a pathogen. Dr. Falkow stated that K-12, in adapting to the laboratory environment, was modified in major ways including modification in the principal outer membrane proteins. He noted that many Escherichia coli strains isolated from healthy individuals could not, anymore so than K-12, be converted into pathogens by laboratory manipulation. He discussed current epidemiological studies which suggest that a few widely disseminated serotypes of Escherichia coli actually cause most cases of disease.

Dr. Baltimore questioned whether an experiment using the HS strain could be related to recombinant DNA experiments employing K-12 host-vector systems. Dr. Gottesman said that the experiments involving Escherichia coli strain HS would be useful, since baseline numbers for mobilization frequencies in the gut would be generated. Dr. Williams said that he agreed with the recommendations advanced by the NIAID Risk Assessment Working Group.

Dr. Susan Wright from the University of Michigan, asked whether Dr. Stuart Levy of Tufts University had looked for the transfer of plasmids from Bacteriodes to Escherichia coli. Dr. Falkow responded that he did not believe that Dr. Levy had. Dr. Young said he was aware of at least three instances in which such transfer had been demonstrated. Dr. Wright questioned whether the Escherichia coli HS strain was proposed for study because industry wants to use it. Dr. Falkow responded that the HS strain was chosen because it was fed to more human volunteers than any other strain and was known to be harmless. He presumed industry would prefer to work with Escherichia coli K-12 as the organism is so well characterized.

Dr. Williams moved acceptance of the NIAID Risk Assessment Working Group report regarding (1) the recommendations on protocols I, II and III, and (2) the proposal to perform risk assessment experiments with Escherichia coli strain HS. The motion was carried by a vote of twenty in favor, none opposed, and two abstentions.

V. PROPOSED EK2 HOST-VECTOR SYSTEMS

Dr. Campbell began the discussion of tab 820 (843/15). He said that Dr. Pouwels of the Netherlands had requested EK2 and possibly EK3 certification of several plasmids derived from plasmid pBR345. He noted that Dr. Pouwels had not specified the host to be used as part of the host-vector system. Dr. Gottesman said that mobilization data should be supplied by Dr. Pouwels. Dr. Campbell questioned the desirability of certifying new EK2 systems at this time. Dr. Gottesman said that in special cases such EK2 systems might be preferable, as the proposed plasmids do not carry antibiotic resistance markers. She said she thought testing to meet EK3 criteria was unreasonable at the present time. She suggested that before the RAC requests additional information from Dr. Pouwels, the Committee should decide whether certifying a new EK2 system is worthwhile. She moved that the RAC continue certifying EK2 systems. This motion was approved by a vote of twenty in favor, one opposed, and two abstentions. Dr. Gottesman then moved that the proposal be referred to the Plasmid Subcommittee to specify the additional data required of Dr. Pouwels, to request the data, to evaluate it, and to report back to the RAC. The motion passed by a vote of twenty in favor, one opposed, and one abstention.

VI. PROPOSED CLASSIFICATION OF PLANT PATHOGENS

Dr. Brill began discussion of tabs 821, 822 and 843/6. He said that Mr. M. T. Goff, U. S. Department of Agriculture, (USDA) and Dr. Philip D. Harriman, National Science Foundation (NSF) requested the inclusion of a proposed Section IV on plant pathogens within Appendix B of the Guidelines. Dr. Brill said that in his estimation this proposed addition does not provide readers of the Guidelines with useful information. Dr. Zaitlin said that the proposal resulted from an attempt to classify plant pathogens on the basis of risk. A Workshop on Risk Assessment of Agricultural Pathogens on March 20-21, 1978, recommended that plant pathogens should be classified on the basis of whether or not they are quarantined. Dr. Zaitlin said the major thrust of the proposal before the RAC was to instruct investigators unfamiliar with plant pathogens to obtain a permit for transporting the organisms. He agreed that the inclusion of the proposed Section IV is not extremely useful, but said no harm would result from including it in Appendix B. Dr. Zaitlin said that minimally a footnote providing instructions on obtaining a USDA permit should be included in the Guidelines.

Dr. Talbot said that Dr. Fredrickson had considered the inclusion of the proposed Section IV of Appendix B in the 1978 Guidelines but decided against this because the rest of Appendix B is merely a republication of classifications previously promulgated in other published documents whereas this is not, and also because the text of the Guidelines does not refer to such a classification, i.e., nowhere in the Guidelines does it matter whether a plant pathogen is "Class 1A" or "Class 1B."

Dr. Tolin said that some plant pathogens are comparable to CDC Class 5 organisms. She added that USDA would like the NIH Guidelines to indicate that regulations concerning such organisms exist. She stated that USDA recommends approval of the proposal as published in the Federal Register.

Dr. Zaitlin moved that those sections of the Guidelines which refer to plant pathogenic organisms should carry a footnote. The footnote would indicate that a USDA permit is required for import and interstate transport of plant pathogens and tell where permits can be obtained. The proposed footnote published in the Federal Register would be the language used. The motion was unanimously approved by a vote of twenty in favor, none opposed.

VII. PROPOSED AMENDMENT OF SECTION III-O

Dr. Gottesman noted that tab 823 (843/3) is a letter from Dr. Stuart Levy of Tufts University School of Medicine. Dr. Levy suggests that

Section III-O specify the use of poorly mobilizable plasmid vectors. Dr. Gottesman noted that most researchers are currently using poorly mobilizable vectors and that specifying use of these plasmids in the Guidelines would not appreciably change the status quo. She stated that implementing this proposal would raise additional questions. Who would determine whether a given plasmid is poorly mobilizable? What procedures and experiments would be required? As the incorporation of this language into Section III-O would entail several procedural difficulties, Dr. Gottesman recommended that Dr. Levy's proposal not be adopted. She suggested instead that a footnote be included in the Guidelines. Dr. Campbell agreed, saying that the Guidelines encourage researchers to use the host-vector system providing the highest containment when these systems are equally appropriate for the purposes of the experiment.

Dr. Gottesman then moved to reject Dr. Levy's suggestion and instead to add the following footnote to Section III-O.

"A subset of non-conjugative plasmid vectors are also poorly mobilizable (e.g., pBR322, pBR313). Where practical, these vectors should be employed."

The motion was carried by a vote of eleven in favor, none opposed, and five abstentions.

VIII. PROPOSED AMENDMENT OF SECTION III-O

Dr. Baltimore said that tab 824 (843/2) is a letter from Dr. Kent Wilcox of the Medical College of Wisconsin suggesting that the term "eukaryotic protein" in Section III-O is ambiguous. Dr. Wilcox suggests that the relevant section of III-O be amended to read as follows:

"* * *An exception, however, which does require prior review and approval by the IBC is any experiment in which there is a deliberate attempt to have the E. coli K-12 efficiently express as a protein the information carried in any gene derived either from a eukaryotic organism or from any virus or viroid which infects a eukaryotic organism.* * *"

Dr. Baltimore said the proposed language is more precise. He questioned the inclusion of "viroids" in this language, but felt that the term could be included for the sake of completeness. He moved acceptance of the proposal as suggested by Dr. Wilcox.

The motion was carried by a vote of eighteen in favor, and none opposed.

IX. PROPOSED AMENDMENT OF SECTION IV-D-2-a

Ms. Cason said that tab 843/1 and 825 is a letter from Mr. David Lester of Princeton, New Jersey, proposing that Section IV-D-2-a, dealing with membership and procedures for the Institutional Biosafety Committee (IBC), be amended so that "non-affiliated members shall be appointed by the governing body of the community in which the institution is located." Ms. Cason said she was unable to support this proposal. While she felt that the public should have some input in the selection process, she questioned the desirability of the community appointing these members. Dr. Talbot noted that two letters had been received during the public comment period, both opposing the proposal.

Dr. Krinsky said that discussion of this issue raises the question of the effectiveness of IBCs. He asked whether IBC function has been evaluated. Dr. Talbot said that a study of IBCs in California has been initiated, and that NIH is considering letting a contract for a larger study, as well as having a meeting of IBC chairmen in the autumn. Dr. Krinsky suggested that the RAC should discuss the issue of assessment of IBCs since IBCs are playing an increasingly important role.

Dr. Ahmed asked whether the current NIH Guidelines stipulate the composition of the local IBC. Dr. Gartland responded that the Guidelines require that at least twenty percent of the IBC membership not be affiliated with the institution. He said the appointment procedure for non-affiliated members differs from institution to institution. He added that the overall composition of the IBC is reviewed by NIH. Dr. Berns said he opposed Mr. Lester's proposal, and raised the question of legal liability. Ms. King noted that legal liability would vary from jurisdiction to jurisdiction and said she opposed mandating what she would consider political representation as opposed to community representation. She supported Dr. Krinsky's proposal that the RAC review the functioning of IBCs.

Dr. Krinsky moved to defer consideration of Mr. Lester's proposal until the RAC had a broader discussion of the effectiveness of local IBCs. Dr. Ahmed questioned the mechanism by which this review would occur. Ms. King suggested that the RAC discuss the issue at the June RAC meeting. She commented on a review of Institutional Review Boards (IRB) in which she had participated, and suggested that a copy of the IRB report be distributed to the RAC as reference material.

Dr. Young suggested it would be prudent to wait for the report of the group studying IBCs in California. Dr. Krinsky asked Dr. Gartland for additional information concerning the California study. Dr. Gartland said that Dr. Diana Dutton's group, which is conducting this survey, is

supported by NSF's Program in Ethics and Values in Science and Technology. He said they had originally intended to survey all American IBCs, but later restricted the study to California. Ms. King suggested that the RAC invite a representative from the California study to address the RAC at the June meeting. Dr. Gottesman suggested that if NIH holds a meeting of IBC Chairmen in the autumn, RAC members could ask them questions at that time.

Dr. Krinsky moved to defer consideration of Mr. Lester's proposal, to schedule a general discussion of IBCs, and to invite a representative from the group doing the California study to address the RAC at the next meeting. The motion was carried by a vote of sixteen in favor, two opposed, and three abstentions.

X. PROPOSED AMENDMENT OF SECTION ON EUKARYOTIC VIRUS VECTORS

Dr. Baltimore briefly summarized the history of the proposal, tab 826 (843/16). Section I-E-5 permits the Director, NIH, to exempt from the Guidelines experiments which do not present a significant risk to health or the environment. Recombinant DNA molecules, of which no component is derived from a eukaryotic virus, and which are propagated and maintained in cells in tissue culture, have been included under this exemption and are cited in Appendix C of the Guidelines. At the December 1979 RAC meeting, Dr. Wallace Rowe proposed that Appendix C be amended to exempt eukaryotic viral fragments of less than one-quarter of the genome. Dr. Setlow appointed a Working Group to study the proposal. The group arrived at the proposal published in the Federal Register (tab 843/16), which would revise Sections III-C-1-(e), ~~III-C-1-e-1~~, III-C-1-e-(1)-(a), and III-C-1-e-(1)-(b) of the Guidelines and add a new Section III-C-1-e-(1)-(c). The proposed new Section III-C-1-e-(1)-(a) reads as follows:

"III-C-1-e-(1)-(a). Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family being considered identical) may be propagated and maintained in cells in tissue culture in the absence of helper virus using P1 containment. The DNA may contain fragments of the genomes of viruses from more than one Family but each fragment must be less than two-thirds of a genome. For such experiments, no MUA need be submitted but prior notice must be given to the IBC as described in Section III-O of the Guidelines. The IBC should handle such registration documents as described in Section III-O."

Dr. Baltimore said that the notion of a viral Family is well defined. A Family is composed of viruses of a common biochemical type. He said that there is no experimental evidence to date that any eukaryotic virus can dispense with one-third of its genetic information and replicate

autonomously or that less than two-thirds of viruses from different Families can fully complement each other's genetic composition.

Dr. Goldstein asked whether the genome of one type of virus could be packaged inside the coat of another virus. Dr. Baltimore replied that transcapsidation between members of two different animal virus Families has never been observed.

Dr. Campbell asked whether low-grade contamination of cells in culture by helper virus is possible. Dr. Dowdle of CDC said that such contamination occurs, generally with viruses being used in the laboratory. Dr. Gottesman questioned whether P1 containment is appropriate for these types of experiments. Dr. Baltimore replied that containment higher than P1 is used in a tissue culture laboratory to prevent contamination of the cell cultures.

Ms. King noted that Dr. Rowe had previously solicited the opinion of several eminent virologists on an earlier version of this proposal, but not on this most recent version. She said she would feel more comfortable if additional comment could be solicited.

Dr. Gottesman then suggested that the RAC vote on each part of the proposal separately. She said the questions which must be addressed when evaluating part one [Sections III-C-1-e, III-C-1-e-(1) and III-C-1-e-(1)-(a) of the proposal] are: (1) Is exemption of two-thirds of a eukaryotic viral genome acceptable in every case; (2) could fragments of viruses of different Families reconstitute to produce a viable virus under these conditions; and (3) what is the probability of adventitious rescue either by cryptic viruses or environmental contaminants. She felt the greatest uncertainty existed concerning the third question and suggested it could perhaps be dealt with either by including a cautioning footnote or by raising containment to P2.

Dr. Baltimore suggested that "in absence of helper virus" be deleted and the following sentence be added to the proposal:

"For such experiments, it must be shown that the cells lack helper virus for the specific Families of defective viruses being used."

Dr. Gottesman suggested that the last two sentences of III-C-1-e-(1)-(a) be struck. This would provide for a more conservative review procedure.

Dr. Gottesman moved acceptance of part one of the proposal as amended. Dr. Setlow then called the vote. This part was approved by the RAC by a vote of thirteen in favor, three opposed, and five abstentions.

The motion as passed by the RAC amends the Guidelines as follows:

"III-C-1-e. All Viral Vectors.

III-C-1-e-(1). Other experiments involving eukaryotic viral vectors can be done as follows:

III-C-1-e-(1)-(a). Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family being considered identical) may be propagated and maintained in cells in tissue culture using P1 containment. For such experiments, it must be shown that the cells lack helper virus for the specific Families of defective viruses being used. The DNA may contain fragments of the genomes of viruses from more than one Family but each fragment must be less than two-thirds of a genome."

During the discussion it was also suggested that in Section III-C-1-e-(1)-(a), after the word "Family," reference to footnote 36 be added, and that footnote 36 be revised to make reference to the third report of the International Committee on Taxonomy of Viruses.

Dr. Gottesman began the discussion of parts two [proposed section III-C-1-e-(1)-(b)] and three [proposed section III-C-1-e-(1)-(c)] of the proposal. They read as follows:

"III-C-1-e-(1)-(b). Recombinants with less than two-thirds of the genome of any eukaryotic virus may be rescued with helper virus using P2 containment if wild type strains of the helper virus are not able to grow in human cells.

III-C-1-e-(1)-(c). Recombinants with less than two-thirds of the genome of any eukaryotic virus may be rescued with helper virus using P3 containment if wild type strains of the helper virus are able to grow in human cells."

Dr. Gottesman said that these sections pertain to experiments in which helper virus is added to cells in culture. She thought limited possibility for transfer exists in these cases: If there were escape into environment the helper and the recombinant defective virus would probably be diluted away from each other leading to a dead end for replication of the recombinant DNA. She noted that this proposal permits a generalized use of viral vectors beyond that which currently exist in the Guidelines. Dr. Baltimore noted that viruses of CDC Class 3, 4 or 5 could not be used as vectors because of Section I-D-1 of the Guidelines. Dr. Gottesman said that a change in the status of Class 3 organisms will soon be considered by the RAC. Dr. Gottesman questioned whether this proposal should be restricted to CDC Class 1 etiological agents.

She asked whether parts two [III-C-1-e-(1)-(b)] and three [III-C-1-e-(1)-(c)] of the proposal would permit the formation of recombinants between viruses of the same Family. Dr. Baltimore replied that it would, but the proposal requires containment levels taken from the current Guideline levels, i.e., P2 containment for viruses that do not propagate in humans and P3 containment for viruses that do. Dr. Goldstein asked how viruses that "are not able to grow in human cells" are defined. Dr. Baltimore replied that this would be tested by growth in human tissue culture cell lines. Dr. Goldstein asked whether antibodies production in people might constitute a more sensitive testing method, as human viruses which are very difficult or impossible to grow in tissue culture do exist. Dr. Baltimore said that viruses that do not grow in tissue culture would not be used as helper virus.

Ms. King asked whether some mechanism exists through which additional comments on these proposals can be solicited from other virologists. She said she would like to see additional justification before evaluating this proposal. Dr. Krinsky agreed.

Dr. Parkinson proposed to defer discussion of the proposal until more information is provided regarding the possible hazards.

Dr. Williams moved that the Committee defer consideration of part two and three of the proposal [proposed Sections III-C-1-e-(1)-(b) and III-C-1-e-(1)-(c)] until the June meeting and request that a summary be prepared of the data supporting these two Sections. The motion was approved by a vote of thirteen in favor, five opposed, and one abstention.

XI. PROPOSAL TO ELIMINATE SECTION I-D-3

Dr. Brill said that Dr. Clarence Kado of the University of California, Davis (tab 827, 843/4) requested that the RAC delete Section I-D-3 from the Guidelines. Section I-D-3 prohibits the deliberate creation of plant pathogens with increased virulence and host range beyond that which occurs by natural genetic exchange. Dr. Brill said that defining virulence and host range is very difficult. He said that virulence can be increased by non-recombinant techniques in the laboratory, but that it is far more difficult to increase the virulence of an organism which can then compete in nature. He pointed out that the use of recombinant technology in plant pathology would facilitate important studies of the basis of pathogenesis. Dr. Zaitlin concurred.

Dr. Krinsky asked whether new information had been advanced to cause plant pathologists to reexamine their ideas on this prohibition. Dr. Zaitlin responded that a workshop had been held in 1978, and the consensus of the participants was that no plant pathogen would be created by recombinant DNA techniques which would be more dangerous than the wild type organisms

themselves. Dr. Tolin said that Section I-D-3 has discouraged progress in this area of research.

Dr. Gottesman said that she saw no reason to continue to prohibit the study of plant pathogens. However, she argued against deleting Section I-D-3 without considering the ramifications on other sections of the Guidelines. She suggested that plant pathogens might be removed from the prohibitions by the same mechanism that will be proposed for CDC Class 3 pathogens. She said the proposal she would be presenting later in the meeting for Class 3 organisms would remove these organisms from the prohibited category and lower review procedures one notch, i.e., experiments involving Class 3 organisms would require prior review by the RAC but would not require prior notice in the Federal Register.

Dr. Gottesman moved that the RAC take no action at this time. Prior to the next meeting a proposal would be published in the Federal Register to delete Section I-D-3 and to consider plant pathogens along the same model to be developed for CDC Class 3 agents.

The motion was accepted by a vote of nine in favor, six opposed, and three abstentions.

XII. PROPOSAL TO INCLUDE ALL SPECIES OF GENUS ERWINIA IN APPENDIX A, SUBLIST A

Dr. Brill presented the request (tab 827, 843/5) from Dr. Clarence Kado of the University of California, Davis to include all Erwinia species in Sublist A of Appendix A of the Guidelines. Currently, only one Erwinia species, Erwinia amylovora, is included on this list of species that exchange DNA by known physiological processes. Dr. Brill said that all the Enterobacteriaceae, which include Escherichia, Shigella, Salmonella, Enterobacter, Citrobacter, Klebsiella, and Erwinia are very closely related. He noted that gene transfers between the Enterobacteriaceae occur frequently. He moved that Dr. Kado's request be approved and that all species of Erwinia be included in Sublist A, Appendix A.

Dr. Gottesman recounted some of the history of the development of Appendix A, and summarized the types of exchanges the RAC previously felt should be demonstrated before an organism is included in Appendix A. Dr. Fredrickson in his Decision Document of December 22, 1978 had accepted as criteria for inclusion in Appendix A,

"Organisms which exchange chromosomal genetic information which becomes stably integrated into the host chromosome" and

"Organisms which exchange chromosomal information that is not necessarily integrated into the chromosome of the recipient (for instance, transfer via F' or R);"

He did not at that time, however, accept

"Organisms which show evidence of a plausible mechanism for exchange (e.g., R⁺ formation or evidence of mobilization of chromosomal genes by an Inc P-1 plasmid)" or

"Organisms which can receive or donate broad host range plasmids."

She questioned whether Dr. Kado's argument of relatedness by 20 percent sequence homology was sufficient to warrant the inclusion of the genus Erwinia in Appendix A.

Dr. Falkow recounted his participation in the development of Appendix A. He said that he believes that chromosomal sequence homology of 20 percent or greater indicates sufficient relatedness to warrant inclusion of organisms on sublists in Appendix A. He said that Erwinia shares a sufficient core of sequence homology with the other Enterobacteriaceae to warrant inclusion in Appendix A, Sublist A. Dr. Young agreed.

Dr. Brill restated his motion to change the listing on Sublist A, Appendix A from Erwinia amylovora to the genus Erwinia. The RAC accepted this recommendation by a vote of fourteen in favor, none opposed, and three abstentions.

XIII. PROPOSAL TO AMEND PORTIONS OF SECTION III-A-2-a

Dr. Nightingale said that she and Dr. Brill had been asked to examine the applicability of the terminology "HV1CV" to host-vector systems other than Escherichia coli K-12. She said a recommendation (tab 843/8) to change the biological containment requirement from HV1CV to HV2 within the subsections of Section III-A-2-a was advanced since it was felt that the CV terminology could not be generalized to host-vector systems other than Escherichia coli K-12. She said that in the EK system, CV containment depends on the plasmid, and the properties of these plasmids can not be generalized to other host-vector systems. She added that in proposing this change she hoped to encourage discussion of the issue. She moved that the proposal be approved as it appeared in the Federal Register.

Dr. Gottesman supported the contention that EK1CV could not be generalized to other host-vector systems. She said that historically the EK1CV nomenclature stood for an EK1 host and a vector certified for use in an EK2 system, and was a position between EK1 and EK2 containment. Dr. Baltimore said the CV nomenclature is meaningless in the yeast system and should be amended. He said HV2 containment is too stringent and suggested that HV1 be substituted for HV1CV. Dr. Gottesman said a proposal substituting HV1 containment for HV1CV would have to be republished in the Federal Register as a proposed major action.

The motion to amend the HV1CV nomenclature of the Guidelines to HV2 was denied by a vote of seven in favor, eleven opposed, and three abstentions.

A proposal to change the nomenclature from HV1CV to HV1 will appear in the Federal Register prior to the June RAC meeting.

XIV. PROPOSED EXEMPTION FOR STREPTOCOCCUS MUTANS AND STREPTOCOCCUS SANGUIS

Dr. Gottesman introduced the proposal of Dr. Francis Macrina of the Virginia Commonwealth University to include Streptococcus mutans and Streptococcus sanguis under the exemption category of Section I-E-4 of the Guidelines (tab 829, 843/9). She said this proposal requests the institution of a new sublist in Appendix A. She said it is a unique proposal in that Dr. Macrina proposes a one-way exemption. Dr. Campbell noted that Dr. Fredrickson, in the decision document accompanying the 1978 Guidelines, specifically endorsed one-way transfer lists in Appendix A.

Dr. Gottesman said the data supplied by Dr. Macrina demonstrate the transformation of Streptococcus sanguis by Streptococcus mutans DNA, but exchange from Streptococcus sanguis into Streptococcus mutans has not been demonstrated. Streptococcus mutans to date appears to be nontransformable. Dr. Gottesman said that the data showing unidirectional transfer is good, transformation occurs at reasonable frequencies and apparently occurs in vivo. Dr. Ahmed asked whether chromosomal gene transfer occurred. Dr. Gottesman said that it did. Dr. Gottesman moved that a new sublist of Appendix A be established for one-way transfer from Streptococcus mutans to Streptococcus sanguis.

The RAC unanimously recommended acceptance of the motion by a vote of nineteen in favor, and none opposed.

XV. PROPOSALS TO CLONE EXOTOXIN A PROTEIN OF PSEUDOMONAS AERUGINOSA

Dr. Broadbent introduced a request (tab 830, 843/10) from Dr. C. W. Shuster of Case Western Reserve University to permit the cloning of the Pseudomonas aeruginosa exotoxin A gene in Escherichia coli K-12. Dr. Broadbent noted that this item had been considered at the December 6-7, 1979 RAC meeting. He said the RAC at that time recommended that the proposal be published in the Federal Register. In the period between the December and March RAC meetings, Drs. James Miller of the University of Louisville and Dr. Stanley Falkow of the University of Washington submitted similar proposals. In support of his request, Dr. Stanley Falkow submitted extensive documentation concerning Pseudomonas aeruginosa. Dr. Broadbent said Dr. Falkow argued that exotoxin A is not a potent toxin in the class of botulinum or diphtheria toxin and is not a primary determinant of disease.

Exotoxin A thus should not be included under the I-D-2 prohibition. Dr. Broadbent said he found Dr. Falkow's argument persuasive. Dr. Young agreed. He said that noncompromised individuals can be colonized by Pseudomonas aeruginosa without mortality. He added that Pseudomonas aeruginosa is a relatively nonpathogenic organism unlike Corynebacterium diphtheriae or Clostridium botulinum in which a single toxin is the determinant of disease. Dr. Falkow said that while exotoxin A is an important virulence determinant of Pseudomonas aeruginosa, the symptoms experienced by infected individuals are not directly related to the toxin per se.

Dr. Campbell asked what degree of containment was used in working with Pseudomonas aeruginosa. Dr. Falkow replied that Pseudomonas aeruginosa is a CDC Class 1 agent.

Dr. Gottesman suggested the RAC first ascertain whether exotoxin A is a "toxin potent for vertebrates" in the context of the Guidelines. Mr. Thornton agreed; he added that the word "potent" should be defined. Dr. Falkow said that exotoxin A is several hundred fold less potent than botulinum and one-fiftieth as potent as diphtheria toxin.

Dr. Young moved that exotoxin A, under the Guidelines, not be considered a potent toxin similar to botulinum toxin. Dr. Campbell noted that the effect of this motion would be to permit exotoxin A to be treated as any other gene of Pseudomonas aeruginosa. He said exotoxin A could thus be cloned into any organism on Sublist A, Appendix A, as an exempt experiment. The RAC accepted the motion by a vote of seven in favor, two opposed, and ten abstentions.

Mr. Thornton suggested it would have been more appropriate to approve the cloning of the exotoxin A gene in Escherichia coli K-12 as requested, rather than approving the broad motion passed. Dr. Campbell agreed.

Dr. Young moved to reconsider the former motion. Dr. Mason agreed, stating that the general issue of what constitutes a potent toxin under the Guidelines should be examined. The motion to reconsider the previous motion was approved by a vote of fifteen in favor, none opposed, and two abstentions.

Dr. Young then made a motion to approve the cloning of the Pseudomonas aeruginosa exotoxin A gene in Escherichia coli K-12, under PI + ERI conditions. The motion passed by a vote of fourteen in favor, none opposed, and three abstentions.

Dr. Setlow later appointed Drs. Broadbent, Maas, and Mason to examine the question of potency of a toxin under the Guidelines.

XVI. PROPOSALS REQUIRING ASSIGNMENT OF CONTAINMENT LEVELA. Request to consider containment appropriate to returning Helminthosporium maydis DNA cloned in yeast to the host of origin

Dr. Zaitlin introduced the request (tab 833, 843/12) of Dr. Olen Yoder of Cornell University to consider the containment level appropriate for the return of Helminthosporium maydis DNA, which had been cloned in Saccharomyces cerevisiae, to the host of origin. Dr. Zaitlin said that there are a number of races of the fungus Helminthosporium maydis, one of which, race T, produces a potent toxin. Certain varieties of corn are susceptible to this fungus. Dr. Zaitlin said that Dr. Yoder intends to study the non-toxin producing race O which is not a pathogen in the U. S. except for the very southern tip of Florida. Dr. Zaitlin recommended approval of the project. Dr. Brill seconded the motion.

Dr. Gottesman asked if Helminthosporium maydis might exchange genetic information with other organisms. Dr. Zaitlin responded that Helminthosporium maydis is a difficult organism to grow and genetic exchange has not been studied.

Dr. Gottesman noted that this experiment returns cloned DNA to the host of origin. She stated that the RAC at some point might wish to consider the generic issue of return of DNA cloned in yeast host-vector systems to the host of origin. Dr. Gottesman said that this issue is complicated by the variety of yeast cloning vehicles, and their behavior in yeast. She said the yeast vector Dr. Yoder proposes to employ recombines into the chromosome. Random pieces of yeast chromosome thus may be picked up by the integrated plasmid. However, she said she felt the proposed experiment to be acceptable at the P2 level requested by Dr. Yoder.

Dr. Goldstein requested that Dr. Zaitlin summarize information about the pathogenicity of this organism. Dr. Zaitlin responded that race T of Helminthosporium maydis is a serious corn pathogen in the United States. He said that race O is not. He pointed out that Dr. Yoder does not propose any experiments with plants, but wishes to study race O under laboratory conditions. Dr. Gottesman asked whether race O ever produces a toxin. Dr. Zaitlin said it apparently does not produce a toxin. Dr. Goldstein asked how race O induces disease. Dr. Tolin responded that race O causes a mild disease by direct action on the leaves in contrast to race T, the toxin of which causes complete collapse of the leaf.

Dr. Goldstein asked whether the lack of health surveillance at Cornell University for experiments conducted at the P1 or P2 levels of containment conforms with NIH policy. Dr. Gartland said the Guidelines mandate

that the institution determine the necessity for health surveillance but do not mandate any required health surveillance program, and that health surveillance varies from university to university.

Dr. Brill moved acceptance of Dr. Yoder's request at the P2 level of physical containment. Dr. Zaitlin absented himself during the vote. The RAC recommended approval of the request at the P2 level of containment by a vote of eight in favor, none opposed, and nine abstentions, including Dr. Zaitlin.

B. Request to evaluate containment appropriate to returning Schizophyllum commune DNA cloned in yeast to the host of origin

Dr. Broadbent said that tab 831 (843/11) concerns a request from Dr. Marvin Schwalb of the New Jersey Medical School to consider containment levels appropriate for the return of Schizophyllum commune DNA cloned in Saccharomyces cerevisiae to Schizophyllum commune. Dr. Broadbent said Dr. Schwalb also requests permission to clone the Saccharomyces cerevisiae derived vector YR414/ura 3 or the Saccharomyces cerevisiae 2 μ plasmid containing Saccharomyces cerevisiae or Schizophyllum commune sequences in Schizophyllum commune. He said Dr. Schwalb is proposing to use P2 physical containment levels. Dr. Broadbent said that Schizophyllum commune is a non-pathogenic basidiomycete which is widespread in nature. He said Schizophyllum commune does not form asexual spores, and forms spores only under easily controllable genetic and environmental conditions. He recommended approval at the P2 level of containment. Dr. Williams and Young concurred, noting that the organism is well controlled under laboratory conditions and that asexual sporulation does not occur.

Dr. Walters pointed out that in a letter of December 13, 1979, Dr. Schwalb suggests that Schizophyllum commune be considered as an HVI system. Drs. Young and Gottesman said that approval for the specific experiment is being considered, and HVI certification of Schizophyllum commune is not under consideration.

Dr. Broadbent moved approval of the proposed experiments at P2 physical containment. The motion passed by a vote of eighteen in favor, none opposed, and one abstention.

C. Consideration of appropriate containment for cloning Wangiella dermatitidis DNA

Dr. Broadbent presented the proposal of Dr. Charles Jacobs of the University of Texas at Austin (tab 832, 860, 843/17). He said Dr. Jacobs requests permission to clone Wangiella dermatitidis DNA in Wangiella dermatitidis using a Saccharomyces cerevisiae/Escherichia coli hybrid plasmid as vector.

Dr. Broadbent said that Wangiella dermatitidis is normally a saprophytic soil organism. In some instances Wangiella dermatitidis is pathogenic and can cause a deep mycosis in humans. He noted that the Center for Disease Control (tab 860) suggests that Wangiella dermatitidis should be handled as a Class 2 agent. Dr. Broadbent recommended P3 containment.

Dr. Mason said the organism can infect normal uncompromised individuals. He said Wangiella dermatitidis has a propensity to invade the central nervous system.

Dr. Nightingale asked if the infection can be treated. Dr. Mason said treatment is difficult and the drugs used are highly toxic. Dr. Young said that he did not believe the proposed recombinant DNA experiment was a hazard, but he was concerned that in dealing with the organism itself deep mycoses might be contracted. He suggested P3 containment.

Noting that Dr. Jacobs proposes to select cycloheximide resistant transformants, Dr. Gottesman asked if cycloheximide would ever be used to treat a Wangiella dermatitidis infection. Dr. Young said it would not.

Dr. Campbell asked if other yeast genes might be linked to the cycloheximide resistance gene. He asked how resistance might be expressed. Dr. Baltimore noted that alterations in membrane permeability are common mechanisms of antimicrobial resistance. Dr. Nightingale asked whether an alteration in membrane permeability might compromise treatment with drugs used clinically to treat the disease. Dr. Gottesman requested that the investigator assess potential alterations in therapeutic drug sensitivity resulting from the introduction of cycloheximide resistance.

Dr. Mason moved to approve the request at P3. Dr. Baltimore asked which plasmid vectors were to be used. Dr. Walters suggested that the investigator be telephoned. Dr. Setlow agreed and postponed the discussion until further information could be obtained.

Following a telephone conversation with Dr. Jacobs, Dr. Milewski reported to the RAC that Dr. Jacobs had agreed to utilize one of the HV2 certified Saccharomyces cerevisiae/Escherichia coli hybrid plasmid vectors and not to select for cycloheximide resistance. Dr. Mason moved approval at the P3 level of containment. The RAC approved the motion by a vote of ten in favor, none opposed, and four abstentions.

D. Request to lower containment for experiments involving a thermophilic Bacillus and the cellulase gene of Sporocytophaga

Dr. Williams introduced a proposal (tab 834, 852, and 843/13) from Dr. David Wilson of Cornell University. He said Dr. Wilson would

like to use a plasmid isolated from Bacillus stearothermophilus or from other thermophilic Bacilli to transfer the cellulase gene from the thermophilic organism Sporocytophaga into a thermophilic Bacillus. Dr. Williams said Dr. Wilson seeks a reduction in containment level from P3 to P2.

Dr. Williams said Dr. Wilson advances two arguments in support of his request:

- (1) Both of the organisms are thermophilic. The optimum temperature for growth of thermophilic organisms is 65 degrees.
- (2) It appears the organisms have a symbiotic relationship.

Dr. Williams said he found the proposal deficient. He said that additional information on the plasmid and the organisms to be employed was required. He moved denial of the request until such documentation is provided. Dr. Young seconded the motion.

Dr. Campbell said that the P3 level for experiments involving non-pathogenic prokaryotes (Section III-B-3 of the Guidelines) was set as an upper limit and not because such experiments were all judged to really require that containment. When data on individual cases indicates a lower containment is appropriate, the level should be lowered.

Dr. Brill said that enzymes of thermophiles almost always function optimally at high temperatures and poorly at 37 degrees C. Dr. Goldstein asked if thermophilic enzymes have no activity at 37 degrees C. Dr. Brill replied that he was not certain activity would be zero at 37 degrees C. Dr. Gottesman said that the extent of symbiosis is not clear; she said the Bacillus apparently grows without the Sporocytophaga in certain circumstances.

Dr. Setlow called the question. Dr. Zaitlin left the room during the vote as Dr. Wilson is affiliated with Cornell University. The RAC denied the request to lower containment from P3 to P2 by a vote of twelve in favor, four opposed, and three abstentions. The RAC requested that additional information be supplied by the investigator.

E. Request to clone the DNA of Schistosoma mansoni

Dr. Maas introduced a proposal (tab 853) from Dr. S. B. Henriques of Brazil to clone the DNA of Schistosoma mansoni. Dr. Maas said the use of recombinant technology to study this organism may result in the production of a vaccine against the parasite. He said Schistosoma mansoni is listed as a CDC Class 3 agent. Dr. Talbot pointed out that under the current Guidelines, a request to study a Class 3 agent must be published in the Federal Register as a proposed major action.

Action on Dr. Henriques' request would have to be deferred until the June meeting.

Dr. Gottesman and Ms. King questioned why an investigator in Brazil would desire an interpretation from the RAC. Dr. Maas suggested that many Brazilian investigators were trained in the United States and would prefer to follow the NIH Guidelines. Dr. Young said schistosomiasis is an important disease affecting millions of individuals in tropical or subtropical regions. He believed the RAC should be responsive to this type of query. Dr. Setlow suggested that Dr. Henriques be queried as to his reasons for submitting this request to the RAC. If appropriate, the issue will be discussed at the June RAC meeting following publication in the Federal Register.

F. Request to construct a plasmid bank for use in Anacystis and Escherichia coli

Dr. Brill introduced a letter (tab 854) from Dr. Louis Sherman of the University of Missouri concerning a project initiated during a sabbatical in Holland.

Dr. Brill said that Dr. Sherman is studying a unicellular blue-green algae, Anacystis, and has successfully transformed this organism with indigenous plasmids as well as with Escherichia coli plasmids. Dr. Sherman intends to construct a plasmid hybrid to be transferred between Escherichia coli and Anacystis. Dr. Brill said that an MUA describing this work had been submitted and that containment levels of P2 + EK2 were indicated in the MUA. He said Dr. Sherman now requests a lowering of containment. Dr. Brill recommended approval of the request at the P1 level of containment.

Dr. Gottesman offered her interpretation of these experiments. She said the transformation experiments into Escherichia coli K-12 which Dr. Sherman described may be performed at P1 + EK0. She said that more complicated experiments would require closer scrutiny. Dr. Brill agreed that Dr. Sherman does not provide a clear idea of future experiments. Dr. Talbot suggested that the investigator be informed by ORDA of what he can do under the Guidelines. Should he desire to do experiments beyond those permitted by the Guidelines, ORDA would suggest he submit details for publication in the Federal Register prior to discussion by the RAC. The members of the committee concurred.

G. Request for an exception to a prohibition in order to study Cauliflower Mosaic Virus aphid transmissibility

Dr. Zaitlin introduced the proposal (tab 857) from Dr. Robert Shepherd of the University of California, Davis. Dr. Zaitlin said he believed this request should be published in the Federal Register as a proposed major action and could not be acted on at this meeting.

Dr. Zaitlin said Dr. Shepherd would like to study that portion of the Cauliflower Mosaic Virus genome which confers aphid transmissibility. Dr. Zaitlin said the Guidelines contain a prohibition against using aphid transmissible strains of Cauliflower Mosaic Virus as recombinant DNA vectors. Dr. Zaitlin said that this stricture against using aphid transmissible Cauliflower Mosaic Virus is inconsistent, as there is no similar prohibition for other viruses.

Dr. Zaitlin said Dr. Shepherd also requests permission to insert that portion of the genome which confers aphid transmissibility into non-aphid transmissible strains. These experiments would increase the virulence or host-range of a pathogen. Dr. Zaitlin said that Cauliflower Mosaic Virus has a very restricted host-range. It attacks certain members of the cabbage Family. In addition, the virus does not replicate in the aphid and thus does not persist in the aphid. He noted that insect-proof cages are used in these studies.

It was agreed that this proposal would appear in a Federal Register announcement as a proposed major action (dealing with both the specific and the generic case) and be discussed at the June RAC meeting.

H. Introduction of rat insulin gene into mouse embryos

Dr. Berns summarized a proposal from Dr. Howard Goodman of the University of California, San Francisco. He said Dr. Goodman proposes to use a defective SV40 genome as a vector to introduce the genes for rat (or human) insulin (or growth hormone) into mouse embryonic cells. The vector will hopefully integrate and rat insulin will be expressed. Dr. Berns said the two cell stage embryos would then be transplanted into a pseudopregnant surrogate mother mouse. These embryos should develop into adult mice producing chemically distinguishable rat insulin. Dr. Berns said Dr. Goodman in his cover letter suggests that this type of experiment could be covered by Section III-C-7-(a) of the Guidelines. In the text of the proposal, however Section III-C-1-(b)-(2) is cited. After further discussion and consideration of these two sections of the Guidelines, a consensus developed among the Committee members that Section III-C-1-(b)-(2) covers this experiment.

XVII. PROPOSAL TO REMOVE CDC CLASS 3 ORGANISMS FROM PROHIBITED EXPERIMENTS

Noting that this proposal must be published in the Federal Register for comment, Dr. Gottesman presented a draft proposal to remove CDC Class 3 organisms from Section I-D-1 of the Guidelines. She said that a working group had been appointed to review the desirability and the possible mechanisms for removing Class 3 agents from the prohibited category.

After some discussion, the working group agreed that Class 3 etiological agents should be removed from the prohibited category. The approach the working group recommends would require RAC review of proposals but would not require prior publication of the proposals in the Federal Register for a comment period.

Dr. Zaitlin said experiments which increase the virulence and host range of plant pathogens currently prohibited by Section I-D-3 could effectively be dealt with under this proposal. Dr. Gottesman agreed to include language concerning plant pathogens in the proposal.

Dr. Krinsky asked about the current status of the pending revision of the CDC classification of etiological agents. Dr. Krause replied that the release date is unknown. Dr. Talbot said that the NIH Guidelines refer specifically to the 1974 CDC Classification of Etiological Agents and that therefore the 1974 edition would be used for the Guidelines until the RAC recommends otherwise.

Dr. Gottesman asked whether the draft language should specify that all work with Class 3 agents would require RAC prior review or should automatically allow it at the P3 level of containment with a lower level possible after RAC review. Dr. Nightingale asked if the number of reviews would be reduced by the inclusion of the P3 containment provision. Dr. Young replied that most facilities studying Class 3 pathogens are equipped with a P3 facility, and he would expect inclusion of the P3 containment level clause to decrease the RAC's burden.

Dr. Walters asked if certain Class 3 agents might be of particular concern. It was noted during this discussion that Smallpox, Whitepox and Alastrim are included among Class 3 agents. Dr. Young and Dr. Berns suggested that mention of these etiological agents in the Guidelines be flagged to indicate that due to World Health Organization efforts at eradication, all activities, including storage of these agents, are restricted to a single national facility.

Dr. Gottesman asked whether the introduction of genomic fragments from Class 3 agents into eukaryotic viral systems might pose any concern. Dr. Berns replied that if less than the whole viral genome is being cloned no special problems should arise.

A proposal covering Class 3 agents will be published in the Federal Register for comment prior to the June meeting, and will be reconsidered then.

XVIII. PROPOSAL ON EQUIVALENCY OF HV SYSTEMS WITH EK SYSTEMS

Dr. Williams presented a proposal submitted by Dr. Richard Novick (tab 828), who was not present at the meeting. He said Dr. Novick has attempted to address the question of whether the Guidelines should be amended to institute complete equivalency between EK and HV host-vector systems. Dr. Williams said he believed Dr. Novick prefers equivalency between the EK systems and the three HV systems certified to date only in shotgun cloning experiments. Dr. Talbot and Dr. Gottesman said they found Dr. Novick's proposal confusing.

Dr. Gottesman cited two major issues on the question of equivalency: (1) Should equivalency be extended to return to host of origin experiments using HV systems; and (2) Should IBCs be authorized to lower containment levels for characterized clones in HV systems, as they were previously permitted to do for EK systems.

Dr. Gottesman suggested that equivalency should not be extended to return to host of origin experiments, i.e., Sections of the guidelines (such as III-C-6) which permit return to host of origin of DNA propagated in Escherichia coli K-12 should not be expanded to also include propagation in other HV systems. She said the characteristics of the certified hosts and vectors vary from system to system, and the general principle should not be applied to all cases. For example, she noted that a wide range of Saccharomyces cerevisiae plasmids exist, some of which carry information which might have an effect on other organisms. In addition, certain yeast plasmids can integrate into the chromosome. She said that for these reasons she views the yeast host-vector systems as more complex than the Escherichia coli systems. She moved that the Guidelines should not be amended to extend equivalency to return to host of origin experiments; rather, each case should be evaluated individually. A straw vote was taken to determine the sentiment of the RAC on this issue.

Dr. Gottesman's proposal was supported by a vote of nine in favor, none opposed, and five abstentions.

A straw vote was then taken to ascertain the sentiment of the RAC on permitting local IBCs to lower containment for characterized clones. The RAC supported this proposal by a vote of nine in favor, one opposed, and three abstentions. A working group composed of Drs. Novick, Brill, Campbell and Gottesman was appointed to develop language for publication in the Federal Register prior to the next meeting.

XIX. PROPOSAL TO INCLUDE SACCHAROMYCES CEREVISIAE UNDER SECTION III-O OF THE GUIDELINES

Dr. Setlow said she had drafted a proposal (tab 855) to include Saccharomyces cerevisiae laboratory strains under Section III-O of the Guidelines. She said additional information concerning yeast has been obtained, including information that Saccharomyces cerevisiae is unable to express higher eukaryotic genes. She said she believed that some of the arguments advanced earlier in support of HVI certification for Saccharomyces cerevisiae could also be advanced to support the inclusion of Saccharomyces cerevisiae under Section III-O. She asked for comments concerning this proposal.

Dr. Gottesman said that the basic characteristics of the Saccharomyces cerevisiae HV systems should be reviewed by the RAC. Dr. Baltimore said that laboratory Saccharomyces cerevisiae strains are as enfeebled as Escherichia coli K-12.

Dr. Gottesman questioned whether the stipulation in Section III-O that any deliberate attempt to express a eukaryotic protein product must be reviewed by the local IBC is sufficient review for experiments using yeast HV systems. Dr. Goldstein said the implications of an Saccharomyces cerevisiae system expressing eukaryotic proteins should be examined in greater depth.

Dr. Young suggested that the data indicating that Saccharomyces cerevisiae systems do not express higher eukaryotic proteins be published in the Recombinant DNA Technical Bulletin. Dr. Goldstein agreed. Dr. Setlow said she would ask the investigators if they would publish this data in the Bulletin. Proposed language including Saccharomyces cerevisiae under Section III-O of the Guidelines will be published in the Federal Register prior to the next meeting.

XX. REPORT OF VISIT TO ELI LILLY AND COMPANY

Dr. Walters reported on the visit of January 28, 1980, which he, Dr. Emmett Barkley, Mr. Ray Thornton, and Dr. Robert McKinney had made to the Eli Lilly plant in Indianapolis, Indiana. Dr. Parkinson asked about Dr. McKinney's background. Dr. McKinney said that he has consulted for the NIH Office of Research Safety for four and one-half years. He possesses a degree in epidemiology with a virology specialty. He had worked at the bench for over 20 years, including 10 years at Fort Detrick where he was a member of the Safety Committee, and had much experience in the design of biological containment facilities.

Dr. Walters said the visit had been arranged by Dr. Barkley at Dr. Fredrickson's request. He said the visit had two goals: (1) to examine the containment facilities at Lilly, and (2) to gather information that would be helpful in revising the draft large-scale standards.

Dr. Walters said the visit consisted of (1) a discussion of review, monitoring, and health surveillance procedures, (2) an inspection of several laboratories and a factory facility, and (3) a discussion of the November 14, 1979, draft large-scale standards.

Dr. Walters reported that the group had seen fermentors of three different sizes: 10-liters, 150-liters and fermentors in the range of 2,000 to 50,000 gallons. He said several potential containment problems must be confronted when dealing with standard fermentors. These include: (1) leakage or spills of inocula introduced or of samples removed from the fermentor for testing, (2) exhausting of aerosols produced during fermentation, (3) leakage of aerosols around the fermentor agitator blade shaft, and (4) the vulnerability of the pipe and valve at the bottom of the vessel.

Mr. Thornton said he wished to add three additional points to Dr. Walters' report. He said Eli Lilly engineers told the group that: (1) most of the supply lines to the facility which contains two 150-liter fermentors were independent of supply lines to the rest of the plant with the exception of electric power and steam lines which were held in common with the rest of the plant, (2) a negative pressure differential within the two 150-liter fermentors ensured that any leakage around the shafts would be siphoned into the fermentors and (3) there were no drain valves at the bottom of these 150-liter units.

Dr. Barkley agreed with the reports of Dr. Walters and Mr. Thornton, and said the group had concluded that the engineering designs employed were appropriate.

Dr. Parkinson asked if the plant was organized. Dr. Johnson replied that the plant is not unionized. Dr. Parkinson asked if members of the group had had the opportunity to speak with workers in the absence of management. Dr. McKinney said he had spoken with two or three people in the absence of management and was very satisfied with their responses to his questions. Dr. Baltimore asked whether the group had spoken with production line workers not necessarily using recombinant methods. Mr. Thornton replied that the group had had the opportunity, but he did not know if anyone had availed themselves of it. Dr. Parkinson asked whether workers are represented on the Health and Safety Committee in the plant, and if the group had spoken with these members. Dr. Johnson replied that there are worker representatives on the Committee, and Dr. McKinney said that the visitors had not spoken with them. Dr. Mason asked about the Eli Lilly employee health and medical surveillance program. Dr. Walters,

Barkley, and Johnson described the program including annual physical examination and serum collection.

Dr. Parkinson asked if any environmental monitoring procedures had been implemented in the plant. Mr. Young replied that during operation of the 150-liter fermentor, a monitoring program measures both the exhaust gases of the fermentors and the room environment twice a week. He said these operations were initiated in October 1979, and no recombinant organisms have been found to date. Dr. Goldstein asked how frequently the fermentors are used. Mr. Young replied that these fermentors were cycled at the rate of four harvests per week. Dr. Young asked if either concentrated air samples or plate samples are examined in the survey. Mr. Young said both types of collection were used. Dr. Baltimore asked how negative pressure in the fermentors was maintained. Mr. Young replied that a vacuum source and a built-in sensor control unit maintain negative pressure.

Dr. Parkinson asked whether worker education manuals dealing with emergencies or with other potential health-related hazards are available in the plant. Dr. Walters said emergency procedures are specified in the documentation provided to the group by Eli Lilly. In addition, a detailed manual for recombinant DNA projects, including emergency procedures, was available.

Dr. Krinsky asked if the group considered it essential that RAC representatives inspect industrial facilities. Mr. Thornton replied he believed it important that the Director of NIH have the right to designate representatives to visit an industrial site. He recommended that this procedure be continued with other large-scale approvals, not that an inspection would necessarily occur in every case, but that NIH has the authority to do so by consent of the company making the application. Dr. Baltimore agreed with Mr. Thornton's position and asked whether the group felt that the large-scale standards should be flexible. Dr. Walters replied that the revision of the draft standards included a shortening and a simplification.

Dr. Goldstein pointed out that a difference between GMAG and the RAC is that GMAG has labor and worker representatives. Dr. Parkinson said those who had gone on the January 28 visit demonstrated naivete of the industrial world, and that he had never yet come across non-unionized workers who are prepared to make aggressive statements about their workplace conditions. Dr. Baltimore said the January 28 visit was not intended to be a regulatory inspection.

XXI. CONTAINMENT STANDARDS FOR LARGE-SCALE RESEARCH AND PRODUCTION

Dr. Walters noted that tab 862 is a revised draft of the proposed physical containment standards for large-scale work. He then introduced Dr. Emmett Barkley who reported on comments received (tab 835-840, 850, 851, 861)

on the earlier (November 14, 1979) draft which had been reviewed at the previous RAC meeting. Dr. Barkley reported that comments on the first draft fell into several broad areas: First, commentators were concerned that procedures for laboratory-scale operations appeared to be extended to large-scale operations. Second, it was suggested that validation procedures be clarified. Third, most commentators placed great emphasis on the containment capability of industrial fermentation vessels. The working group, therefore, attempted to treat the closed fermentation system as the fundamental aspect of containment in large-scale operations and attempted in its November 1979 draft to stipulate only two levels of containment, P2-LS and P3-LS. In response to comments, the working group subsequently concluded that establishing a P1-LS containment level was appropriate. A fourth area addressed by commentators was the monitoring of containment systems. The working group felt it appropriate to establish monitoring requirements on systems that are actually employed. Fifth, the working group also recognized that more attention should be directed to health surveillance. The working group has recommended that health surveillance requirements be a specific recommendation for the P3-LS level.

Dr. Walters suggested that the revised large-scale physical containment standards be published in the Federal Register for an additional period for comment.

Dr. Krinsky said he would present what might be termed a minority report. He said that in his experience with regulation of new technologies at the level of production, the subpopulations at the greatest risk have little or no input into the decision-making process. In his view, it is the moral responsibility of those determining industrial standards to actively seek input from subgroups at potentially greater risk. Yet, his suggestion of bringing representatives of labor before the RAC was denied. He said that the revised standards do not reflect a sufficiently deep analysis of health surveillance. He felt strongly that the Committee should not be involved in what is tantamount to certifying industrial activities, but if the RAC is to be involved, labor should be represented and the RAC should interact directly with the Industrial Practices Subcommittee of the Federal Interagency Committee which has OSHA and NIOSH expertise.

Dr. Young said he was concerned that the RAC is moving more towards a regulatory than an advisory mode. He said he believed the role of the RAC should be to be advisory on scientific principles. He expressed concern that the RAC would become a forum in which industrial procedures are "certified."

Dr. Goldstein said he believed the RAC has exceeded its mandate; in a de facto fashion the RAC is regulating the private sector. He said the RAC does not possess the expertise to evaluate production aspects.

Dr. Williams favored the RAC continuing to play an advisory role in developing large-scale Guidelines, but felt uncomfortable in assessing individual proposals from industry.

Dr. Walters said he believed a need exists for interim standards in an area where no standards currently exist. Dr. Baltimore said he believed the RAC has the responsibility of overseeing the development of recombinant DNA technology. He said the RAC fulfills this function by providing an overview of safety questions. He stressed the time required by OSHA and NIOSH to develop and implement regulations. He congratulated the large-scale working group on proposing an approach relevant to the industrial situation.

Dr. Mason said the taxpayers supporting research expect to obtain some applied benefit from this research. He said the application of recombinant technology to benefit people should not be impeded by a lack of guidelines. He said he agreed that the composition of the RAC is not optimal to evaluate industrial scale-up, but felt that application of the technology should not be delayed because no group is prepared to provide guidelines or regulations. Mr. Thornton said the RAC either possesses, or should be able to obtain, competent advice in industrial application of recombinant technology. Dr. Parkinson said that OSHA possesses the competence and the mandate to deal with this area. Dr. Gottesman suggested that the RAC continue to review submissions for the characterization of clones and to evaluate potential hazards. She said that other procedures could be established for reviewing other aspects of industrial submissions.

Dr. Setlow called on Dr. Christine Oliver of the Oil, Chemical and Atomic Workers International Union to address the RAC. Dr. Oliver said she would reiterate some of the concerns she had expressed in a letter to Dr. Setlow. She said she believed the responsibility for regulating the industrial application of recombinant DNA technology lies with OSHA and NIOSH. The implementation of guidelines for large-scale recombinant DNA technology require a more adequate understanding of the workplace. It is very difficult to visit a plant and obtain any idea of working conditions without an in-depth discussion with the workers themselves. In labor's experience, containment is difficult to attain. As production is scaled up equipment breakdown becomes more frequent. As overtime increases, the number of accidents increase and the subsequent exposure of workers increases. She offered the expertise of her union to the RAC and to OSHA and NIOSH.

Dr. Johnson of Eli Lilly and Company then briefly addressed the RAC. He said that while the RAC may not necessarily have the responsibility of regulating the private sector, the Committee does have the responsibility of overseeing recombinant DNA research regardless of how it is supported. He said he hoped that the RAC will continue an interim approach to industrial projects as jurisdictional authority is sorted out. He commended

the working group on the large-scale standards saying they are consistent with good laboratory and good manufacturing practices. He made two suggestions concerning these standards: (1) that "non-debilitated" be defined, and (2) that negative pressure should be required at the P3-LS level rather than at P2-LS.

Mr. Myers of NIOSH said that NIOSH and OSHA are responsible for regulating the workplace. He reported that these two agencies are cooperatively establishing a process in this area. He said these agencies view the RAC as a valuable source of information in the establishment of recommendations. Mr. Pauker of NIOSH noted that all of NIOSH's comments on the earlier draft of the large-scale standards had not been adopted, and that the section on health surveillance is still not to the satisfaction of NIOSH. Mr. Thornton commended the working group and moved that the proposed large-scale standards be published in the Federal Register as recommended Guidelines. Dr. Harris said she supported the motion noting that these standards provide a framework for industry. The motion was passed by a vote of sixteen in favor, two opposed, and one abstention.

Dr. Krinsky then moved that (1) the RAC review only large-scale projects involving no proprietary information at NIH-funded institutions, and (2) the RAC refer other large-scale proposals that do not fall into the above category, with RAC recommendations, to OSHA. Dr. Krinsky said that in this manner the RAC could review the material but would not certify either facilities or proposals. Dr. Baltimore moved to table this proposal. He said he believed the Committee's sentiment was that the RAC should continue to handle these applications and that OSHA was not prepared to handle such proposals at present. The RAC passed the motion to table by a vote of eight in favor, six opposed, and four abstentions.

XXII. PROPOSED LARGE-SCALE EXPERIMENT

Dr. Gottesman introduced the proposal (tab 841) from Dr. Benjamin Hall of the University of Washington. She said that Dr. Hall would like to grow, on a large scale, Saccharomyces cerevisiae carrying a Saccharomyces cerevisiae/Escherichia coli hybrid plasmid containing a Saccharomyces cerevisiae gene. She said this proposal had been considered at a previous meeting but disapproved as the request lacked information on the fermentors. Genentech, Inc., had agreed to grow the cultures for Dr. Hall, but their 75-liter fermentation system had not at that time been evaluated by the RAC.

Dr. Gottesman said she had voted at the previous meeting to approve this proposal as she viewed the experiment as essentially self-cloning. Dr. Walters asked Dr. Gottesman if this experiment could be considered

exempt from the Guidelines. Dr. Gottesman responded that the yeast plasmid carries a fragment of Escherichia coli DNA and therefore cannot be considered exempt.

Dr. Gottesman recommended that the RAC approve the proposal and so moved. Dr. Young agreed and seconded the motion. Dr. Young said that the yeast sequence is a well-characterized sequence in a well-characterized vector.

The RAC passed this motion by a vote of twelve in favor, none opposed and three abstentions.

XXIII. CLOSED SESSIONS

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

XXIV. FUTURE MEETING DATES

The RAC selected the following dates for future meetings:

June 5-6, 1980

September 25-26, 1980

XXV. ADJOURNMENT

The meeting adjourned at approximately 5:15 p.m. Friday, March 7, 1980.

Respectfully 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.

July 8, 1980
Date

Jane K. Setlow
Jane K. Setlow, Ph.D.
Chairman
Recombinant DNA Advisory Committee
National Institutes of Health