

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

1014

RECOMBINANT DNA ADVISORY COMMITTEE

MINUTES OF MEETING¹

JANUARY 8-9, 1981

The Recombinant DNA Advisory Committee (RAC) was convened for its twenty-first meeting at 9:00 a.m. on January 8, 1981, in Conference Room 10, Building 31C, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20205. Mr. Ray Thornton (Chairman), President, Arkansas State University, presided. In accordance with Public Law 92-463, the meeting was open to the public from 9:00 a.m. to 3:00 p.m. on January 8, and from 9:00 a.m. to adjournment on January 9. The meeting was closed to the public from 3:00 p.m. to 6:00 p.m. on January 8 for the review of proposals involving proprietary information.

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

A Committee roster is attached. (Attachment I)

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

Dr. Charlotte Bell, U. S. Department of Justice; Dr. Howard Berman, U. S. Veterans Administration; Dr. Donald DeVincenzi, National Aeronautics and Space Administration; Dr. George Duda, U. S. Department of Energy; Dr. Timothy J. Henry, Food and Drug Administration; Dr. Herman Lewis, National Science Foundation; Dr. Chia T. Chen, OSHA, U. S. Department of Labor; Dr. Sue Tolin, U. S. Department of Agriculture; and Dr. William J. Walsh, III, U. S. 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. Marilyn Bach, NIAID; Dr. Stanley Barban, NIAID; Dr. W. Emmett Barkley, ORS; Mrs. Betty Butler, NIAID; Ms. Mary Donovan, NIAID; Dr. John Irwin, ORS; Dr. Richard Krause, NIAID; Dr. Elizabeth Milewski, NIAID; Dr. Stanley Nagle, NIAID; Dr. John Nutter, NIAID; Dr. Bernard Talbot, OD; and Dr. Rudolf Wanner, ORS.

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

Dr. E. A. Agostini, Pfizer, Inc.; Dr. Ray Berger, Schering-Plough Corp.; Ms. Irene Brandt, Eli Lilly & Co.; Dr. Peter Bostock, New Brunswick Scientific Co.; Dr. Jerry Callis, U. S. Department of Agriculture, Plum Island; Dr. Aileen Compton, Smith-Kline & French; Mr. L. Curley, New Brunswick Scientific Co.; Dr. Mark Finkelstein, Schering-Plough Corp.; Dr. Patrick Gage, Hoffman LaRoche, Inc.; Ms. Lizabeth Gelber, Bokon Productions; Dr. Jean Gudas, University of California, Los Angeles; Dr. Paul Hung, Abbott Research Laboratories; Dr. James Hunt, Chemapec; Dr. Dorothy Jessup, U. S. Department of Agriculture; Dr. Attila I. Kadar, Food and Drug Administration; Mr. W. H. Kampen, New Brunswick Scientific Co.; Mr. Geoffrey Karny, Office of Technology Assessment; Dr. Paul Leibowitz, Schering-Plough Corp.; Ms. Carter Leonard, Blue Sheet; Mr. Ronald Leonardi, KABI Group, Inc.; Mr. Charles Marwick, Medical World News; Dr. James McCullough, Library of Congress; Mr. Bing Miller, New Brunswick Scientific Co.; Dr. Henry Miller, Food and Drug Administration; Dr. Philip Miller, Hoffman LaRoche, Inc.; Dr. Ann Norberg, Monsanto Co.; Mr. Seth Pauker, National Institute for Occupational Safety and Health; Dr. Stephen Pijar, Food and Drug Administration; Dr. Vishva Rai, Hoffman LaRoche, Inc.; Dr. Michael Ross, Genentech, Inc.; Mr. Dan Smith, Peoples Business Commission; Mr. Charles Turbyville, Genetic Engineering Letter; Dr. Marvin Weinstein, Schering-Plough, Corp.; Dr. Susan Wright, University of Michigan; Dr. Bill Young, Genentech, Inc.; and Dr. Robert Zaugg, Teknekron, Inc.

I. CALL TO ORDER AND OPENING REMARKS

Mr. Ray Thornton, Chairman, called the meeting to order at 9:00 a.m., January 8, 1981. He introduced two newly appointed members of the committee: Dr. King Holmes of the Division of Infectious Diseases of the U.S. Public Health Service Hospital, Seattle, Washington and Dr. Robert McKinney of the Division of Safety of the National Institutes of Health (NIH).

Mr. Thornton said he had asked Dr. McKinney to serve as co-chairman, with Dr. Berns, of the newly instituted Large-Scale Review Working Group. Mr. Thornton said that any RAC member interested in serving on that working group should contact him.

Mr. Thornton announced that agenda items scheduled for Friday morning would be considered on Thursday if time permits.

II. MINUTES OF THE SEPTEMBER 25-26, 1980 MEETING

Dr. Harris reviewed the minutes of the September 25-26, 1980 RAC meeting (tab 978) and said she found them to be correct. She moved for adoption, which was seconded by Mrs. Cason. Dr. Susan Wright cited an exchange between herself and Dr. Maxine Singer at the September meeting concerning the composition of biohazard committees in the United Kingdom. Dr. Singer had drawn a parallel between the situation regarding recombinant DNA in the U. S. and in the U. K. Dr. Wright had said that the analogy was inaccurate because genetic engineering is regulated in the United Kingdom, i.e., the laboratories are inspected and the composition of the local

Biohazards Committee is more stingently controlled. Dr. Singer replied that she had not intended to draw a parallel. Dr. Wright requested that this exchange be included in the minutes. She also requested that comments by Dr. Krinsky on the survey of California IBCs be included. She said following the report on the California IBC survey, Dr. Krinsky stated that the kind of evidence the RAC was preparing to accept in the social sciences was not of the high quality that RAC members would normally expect in their own areas of expertise. Mr. Thornton recommended that those comments be included in the minutes of the January 8-9, 1981 meeting and that the minutes of the September 24-25, 1980 meeting be approved as moved and seconded.

Dr. Maas pointed out some typographical errors in the draft minutes. The minutes, with the suggested amendments, were approved by a vote of 17 to 0.

III. MEETING OF INSTITUTIONAL BIOSAFETY COMMITTEE CHAIRPERSONS

Dr. Krause, before reporting on the IBC Chairperson's meeting (tab 977), briefly commented on the February 3-5, 1981 meeting of the U.S. - Japan Cooperative Program for Recombinant DNA Research. He said this meeting will focus on the guidelines for research, on host-vector systems, and on risk assessment studies.

Dr. Krause noted the first IBC Chairperson's Meeting had been held two years ago. Since that meeting, the Guidelines had evolved markedly with greater responsibility being delegated to the local IBC. The

IBC Chairperson's Meeting sponsored by the National Institutes of Health on November 24-25, 1980, was an attempt to identify problems the IBCs might be encountering. In addition, the conference was viewed by NIAID as the first stage in a possible formal evaluation of the functioning of IBCs.

Two hundred and twelve individuals attended the meeting, including representatives from 154 IBCs. Among the participants were 21 individuals from the industrial sector and 5 IBC community members. Four current RAC members, Dr. Krinsky, Ms. King, Dr. Mason, and Mr. Thornton, and some former RAC members, participated. In a panel on the operation of IBCs, three IBC Chairpersons, Dr. Patrick Gage of Hoffman-LaRoche, Dr. Alan Garber of Baylor College of Medicine and Dr. Melvin Chalfen of Massachusetts Institute of Technology, addressed problems which would be examined in conference workshops, and Mr. Robert Spanner evaluated IBC function from the vantage point of a community member. Three workshops subsequently addressed problems associated with (1) the IBC as a means of implementing institutional oversight, (2) health surveillance, monitoring and certification, and (3) procedures and operations. Reports from the workshop leadership teams, composed of a RAC member, an IBC chairperson and an NIH staffer, were presented at a plenary session the following morning. (A transcript of the plenary session appears under tab 977). At this session several recommendations were voted. One of these recommendations is that experiments currently covered by Section III-0 should be exempted from the Guidelines.

Dr. Krause said many of the chairpersons believed much had been achieved at the meeting. As a consequence of this view, of concerns expressed for other safety issues, and of a vote taken at the IBC Chairpersons meeting, NIAID was reconsidering the IBC evaluation plan. NIH is tentatively planning to broaden the scope of the evaluation to include safety matters other than recombinant DNA issues.

Dr. Krinsky said the transcript of the plenary session (tab 977), accurately reflected the tone of the meeting. He said that one point of disagreement among the participants was on the question of whether there should be biosafety committees, or whether their functions could be performed better by some other method.

Dr. Mason commented on the health surveillance, monitoring and certification workshop. He said there had been a great deal of discussion on the cost/benefit ratio of health surveillance programs. The consensus was that if unusual medical surveillance were to be required, it should be well defined and carefully controlled in order to obtain reasonable data. No workshop participant felt there was justification for such an in-depth program; but to do less, if one really was worried, was not sensible. Most participants felt heavy stress should be placed on education in good laboratory practices and procedures for all potentially hazardous work. To single out recombinant DNA for special emphasis was unwarranted.

Dr. Baltimore asked if positive support for any part of the Guidelines had been evidenced at the meeting. Mr. Thornton replied that participants

indicated that the Guidelines reflect a consensus hammered out between science and society.

Mr. Thornton then summarized his impression of the meeting. He felt most chairpersons recognized the value of IBCs, but were concerned that the focus of those efforts was recombinant DNA activities. They did not feel one area of research should be singled out for special attention. A second concern was the "paperwork burden" associated with the Guidelines. A recommendation made by the Chairpersons to exempt experiments covered by Section III-O of the Guidelines derives from this concern. Dr. Mason offered his perception that many IBC chairpersons did not like the formality associated with the review procedures and the current structure of the IBC.

Dr. Gottesman suggested three mechanisms for alleviating some of the "paperwork burden": (1) Exempt those experiments falling under Section III-O; or (2) Require P1 + EK1 containment conditions but dispense with the requirement for registration documents; or (3) Require the IBC to maintain a registry, but not to review the experiments. In the latter case, there could be a requirement for some Institutional officials, but not the full IBC to review the documents. She said she herself could not support the first option. She supported the second or third options with the understanding that large-scale (i.e., greater than 10 liters) applications still be considered as a special case.

Dr. Baltimore said he would support a motion to exempt experiments covered by Section III-O from the Guidelines, including large-scale experiments. He suggested consideration be given to changing RAC to a general advisory committee on biosafety. He felt there were many biosafety issues other than recombinant DNA which deserved consideration. Dr. Mason concurred with Dr. Baltimore's views.

Dr. McGarrity asked Dr. Barkley of the NIH Division of Safety to comment on the current status of federal guidelines on chemical carcinogens and on etiological agents. Dr. Barkley replied that the CDC guidelines on etiological agents, which are intended as a voluntary code of good practice, have been issued in draft form for comments from the scientific community.

Dr. Barkley said the Occupational Safety and Health Administration (OSHA), responsible for promulgating standards to protect American workers from exposure to chemical carcinogens, is considering the problem of carcinogen use in research laboratories. One method of addressing the problem would recognize the use of informed judgement by principal investigators. The Department of Health and Human Services (DHHS) is developing guidelines on control of carcinogens, which will apply specifically to DHHS intramural laboratories. Guidelines for the use of chemical carcinogens in NIH intramural laboratories will be issued soon.

Dr. Barkley said aspects of new regulations of the Environmental Protection Agency (EPA), particularly with respect to the Resource Conservation and

Recovery Act, were also reviewed at the IBC Chairperson's meeting. These regulations are an attempt to reduce the indiscriminate disposal of toxic chemical waste in the environment.

Dr. Berns said he found the CDC's proposed biosafety guidelines for etiological agents to be capricious and unscientific, and the CDC unresponsive to expressed concerns. He admitted that the guidelines may be de jure, voluntary, but feared they would not be voluntary, de facto.

Dr. Williams, while admitting that the CDC guidelines and OSHA regulations were important, urged that the RAC resist the temptation to address a variety of issues beyond its charge. Mr. Thornton supported this position.

Mr. Thornton asked for a straw vote to gauge RAC sentiment. He first asked how many RAC members preferred to maintain the status quo regarding Section III-O. No one favored this approach. He then asked how many favored doing something to materially reduce or eliminate the paperwork and reporting functions for experiments covered by Section III-O. Nineteen individuals supported this position. He then asked how many members felt serious consideration should be given to exempting entirely from the Guidelines experiments currently covered by Section III-O. Ten members supported this position. Mr. Thornton asked NIH staff to prepare language on a series of options for publication in the Federal Register prior to the April meeting.

Dr. Campbell suggested that experiments currently covered by Section III-O be exempt from the Guidelines, but the Guidelines include a recommendation that these experiments be done under P1 containment conditions.

Dr. Krimsky asked for a clarification of the mechanism by which funds are earmarked for evaluations. Dr. Talbot replied that Congress appropriates budgetary funds for each Institute of the National Institutes of Health. A separate law specifies that up to one percent of those funds may be allocated to evaluation. A detailed review procedure within DHHS is used to allocate these funds among specific evaluation projects.

IV. UPDATE OF PROPOSED RISK ASSESSMENT PROGRAM AND REVIEW OF PAPER ON E. COLI POPULATIONS

Dr. Williams reviewed the Proposed First Annual Update of the Program to Assess Risks of Recombinant DNA Research (tab 962). Dr. Williams highlighted some of the items discussed in the plan, including the protocols that originated in the Falmouth Workshop concerning colonization by E. coli K-12 and transmission of genetic information from E. coli K-12 to the intestinal flora, and the protocol involving the E. coli strain HS, a good colonizer, and plasmid pBR325. The plan also discusses the results of the polyoma experiments which attempted to determine if recombinant organisms containing oncogenes would induce tumors, and includes a summary of the Pasadena Risk Assessment Workshop held on April 11-12, 1980. NIAID has awarded a contract to the University of Minnesota to

develop a course on basic microbiological practices and techniques for work with hazardous agents.

Dr. Krinsky noted the statement in the plan that "no risks of recombinant DNA research have been identified that are not inherent in the microbiological and biochemical methodology used in such research." He asked whether an increase in host range resulting from a recombinant manipulation would be considered a counter-instance to that statement. Dr. Krause replied that the risk assessment plan indicates that no such case has been known to occur; it does not imply that such an event is an impossibility.

Dr. Krinsky asked if the results of EPA contracts would be available to RAC, and if these results would be integrated into future NIH risk assessment analyses. Dr. Talbot replied that EPA reports periodically to the Industrial Practices Subcommittee of the Federal Interagency Committee. Minutes of these meetings are forwarded to RAC. He noted that the USDA may also perform some risk assessment studies and the NIH will be kept abreast of those studies.

Dr. Krinsky raised a point regarding the wording in the second paragraph, third column on page 61876, dealing with colonization of the intestinal tract. It was agreed that the word "known" should be deleted from the last clause of the paragraph, which would now read as follows: "... even though E. coli K-12 has apparently lost those characteristics that are required for colonization of the normal intestinal tract." Other

questions by Dr. Krinsky regarding the plan were answered by Dr. Krause and RAC members.

Dr. Wright suggested the risk assessment plan should be footnoted and referenced, and the identities of investigators disclosed. Dr. Wright also felt that the controversy between Rowe-Martin and Rosenberg-Simon on the interpretation of the Rowe-Martin polyoma experiments should be included in the document. Dr. Krause, Dr. Williams and Mr. Thornton noted the debate was presented to, and carefully considered by, the RAC. Dr. Baltimore said that he does not believe there is a serious controversy concerning the interpretation of the polyoma experiments; rather, there is only what he considers a twisted interpretation of those experiments by some people. Dr. Krinsky stated his understanding that any positive results in the polyoma protocols would be very important and said that the reviewers who accepted the Rosenberg-Simon article for Nature must have seen some value in the article. [Executive Secretary's note: Nature has confirmed that the Rosenberg-Simon article appeared as a "feature article" rather than as a "scientific paper" and therefore was not formally peer reviewed]. Dr. Campbell said that what Rosenberg and Simon judged to be positive results were what Rowe and Martin considered the controls for the experiment.

Mr. Pauker asked if studies to elucidate aspects of survival and colonization of different strains of E. coli would be undertaken. Dr. Krause pointed out that the plan states that NIAID has awarded a grant to an investigator to study the molecular mechanisms of E. coli colonization.

specifically the relative importance of plasmid or chromosomal determinants of colonization. Dr. Levine said this field is very fertile and developing very rapidly.

Mr. Thornton called on Dr. Holmes to review tab 963, a paper entitled "Genetic Diversity and Structure in Escherichia coli Populations."

Dr. Holmes said that in this article investigators surveyed twenty enzymes from one hundred nine clones of E. coli. In addition to the wild type isolates, twenty-four laboratory K-12 strains were studied. The genetic diversity the investigators observed led them to conclude that recombination in nature, of the genes coding for the studied proteins, is rare.

One of the E. coli isolates had been obtained from an infant in a Massachusetts hospital nursery. The twenty assayed enzymes from this strain were indistinguishable electrophoretically from the same twenty enzymes in laboratory strain E. coli K-12. Dr. Krinsky said he asked that this be discussed, as he wondered whether it might be indicating that E. coli K-12 is surviving in nature. Drs. Levine, Campbell and Nightingale all said that just because the Massachusetts nursery isolate has 20 enzymes electrophoretically identical to E. coli K-12, does not at all mean that it will resemble E. coli K-12 in the parameters that are important for E. coli K-12's lack of ability to colonize. It was agreed that the data on the 20 enzymes are "a small drop in a large bucket;" very incomplete data towards establishing the similarity of the Massachusetts isolate to E. coli K-12. Further it was pointed out

that the paper states the Massachusetts isolate differs in its bacteriophage sensitivities from E. coli K-12.

V. REVISED GENETIC MANIPULATION ADVISORY GROUP GUIDELINES

Dr. Gottesman began discussion of the United Kingdom's revised Genetic Manipulation Advisory Group (GMAG) guidelines for recombinant DNA experiments (tab 964). She said the British had instituted a system in which numbers are assigned for "access," "expression," and "damage." The numbers are multiplied together to obtain a final figure which determines the recommended physical containment level. She said the RAC applies similar principles when evaluating recommended containment, but in not as explicit a form. GMAG, like the RAC, has been delegating increased responsibility to local committees. She said she did not perceive any significant differences in approach between the U. S. and the British situation, which would compel the RAC to take action.

Mr. Pauker pointed out that GMAG still requires some central notification and registration. Dr. Wright said the GMAG guidelines generally required higher containment levels than the NIH Guidelines. Others said that the latest GMAG revision seemed to lead to generally lower containment levels in the U. K. as compared to the U. S.

VI. PROPOSED PROCEDURES FOR MINOR MODIFICATIONS OF PREVIOUSLY APPROVED LARGE-SCALE RECOMBINANT DNA EXPERIMENTS

A. Application Procedures for Minor Modifications of Previously Approved Large-Scale Recombinant DNA Experiments.

Dr. Gottesman began discussion of the proposed application procedures for minor modifications of previously approved large-scale recombinant DNA experiments (tabs 965, 976/3). Dr. Gottesman said that currently all large-scale experiments are reviewed by the full RAC; the proposal is an attempt to develop an expedited procedure for minor modifications of previously approved large-scale experiments. The proposal provides a procedure for determining whether a modification is minor. A request for evaluation of a minor modification would be sent to ORDA. If ORDA believes it is a minor modification, the request will be sent to a working group composed of at least two RAC members. If possible, these members should have participated in the review of the original approval. If the working group unanimously agrees that the modification is minor and that the changes do not significantly alter the organism in a way that is likely to affect containment of the organism or the vector, or the nature of the expressed product from that presented originally to RAC, recommendation for approval will be transmitted to ORDA. If the working group does not so find, the proposal would be presented to the full RAC for consideration. Dr. Gottesman moved acceptance of the proposed language. Dr. Berns seconded the motion.

Dr. Goldstein suggested that each proposal be circulated to all RAC members at the time that it is sent for review to the working group. This would provide an opportunity for all RAC members to comment. He further suggested that the decision of the working group be transmitted to all RAC members. Dr. Gottesman suggested instead that a summary of minor modifications approved between meetings using the minor modification working group procedure be provided to RAC at each RAC meeting. Dr. Goldstein accepted this proposal, and it was agreed that ORDA would provide such a summary.

Dr. Leibowitz of Schering-Plough Corporation suggested that certain minor modifications of previously approved large-scale experiments using E. coli K-12 host-vector systems might be approved by the local IBC. Dr. Gottesman said she preferred the minor modification procedure as in tab 976/3. Dr. McKinney agreed. By a vote of seventeen in favor, none opposed, the RAC accepted the proposed language (976/3).

B. Proposed Procedures for Change of Locale of Previously Approved Large-Scale Recombinant DNA Experiments.

Dr. Gartland posed the question of how to process changes of site for large-scale production using previously approved clones, i.e., a clone which had been approved for scale-up at one site would be moved to another physical facility. A second IBC might then be charged with oversight responsibilities.

Dr. Baltimore, noting that the RAC no longer reviews physical facilities, felt changes of locale were therefore not appropriate RAC considerations. It was agreed that ORDA would administratively process such proposals.

[Executive Secretary's Note: The following statement dealing with changes of facility has been added to the "Application Procedures for Large-Scale Recombinant DNA Experiments":

"7. Should a clone, previously approved for scale-up at one facility, be proposed to be moved to a second facility, the IBC with oversight responsibility at the second site shall submit to ORDA a registration document and receive ORDA approval, prior to initiating scale-up. No RAC review would be required."]

VII. PROPOSED CONTAINMENT FOR EXPERIMENTS INVOLVING NONPATHOGENS

Dr. Brill introduced the proposal (tabs 966, 973, 976/2, 980, 981, 982, 983, 984, 985, 987, and 988) to amend the Guidelines to permit use of nonpathogenic prokaryotes and nonpathogenic lower eukaryotes as donors and recipients in recombinant DNA experiments under P1 containment conditions. Specifically, it was proposed in the Federal Register on November 28, 1980, that a new Section, III-0-2, would be added to the Guidelines as follows:

"III-0-2. Experiments Involving Nonpathogenic Prokaryotes and Lower Eukaryotes. Recombinant DNA experiments involving prokaryotes and

lower eukaryotes, nonpathogenic [2A] for man, animals, or plants, can be conducted under P1 containment."

Changes were also proposed in other sections of the Guidelines to accomplish this change.

In discussing the proposal, Dr. Brill suggested that this proposed language be amended as follows: (1) following the word "plants", the words "and only DNA from such sources" would be added; and (2) a requirement would be added that the experiments, and documentation that the organisms are not known pathogens, must be registered with the local IBC; and (3) containment for these experiments would be raised from P1 to P2. It was noted that Dr. Novick, in a letter, had suggested that containment be raised to P2. Dr. Brill moved acceptance of the proposal as amended. Dr. Fedoroff seconded the motion.

Dr. Goldstein asked what type of documentation would be submitted to the local IBC concerning nonpathogenicity. Dr. Brill replied that the investigator should provide evidence from the literature that the organism is not pathogenic.

Dr. Berns said that the proposed requirement for P2 had no scientific basis and moved to amend the language to require P1 containment.

Drs. Brill and Fedoroff accepted Dr. Berns' amendment.

Dr. Berns proposed an amendment to Dr. Brill's amendment to change the word "registered" to the word "reviewed". Drs. Brill and Fedoroff agreed.

Mr. Thornton asked Dr. Brill to restate his proposal as amended.

Dr. Brill read the following amended motion:

"Recombinant DNA experiments involving prokaryotes or lower eukaryotes nonpathogenic to man, animals, or plants, and only DNA from such sources, can be conducted under P1 containment conditions. The experiments must be reviewed by the local IBC with documentation that the organisms are not known pathogens."

Dr. Gottesman said she could not support the proposal as: (1) It covers an enormous variety of organisms, (2) It does not restrict the type of vector to be used (e.g., conjugative plasmids would be permissible), and (3) "Nonpathogenic" may be defined differently by different IBCs. This proposal would represent a departure from current Guideline philosophy. Dr. Gottesman agreed, however, that an expedited procedure for evaluating containment for experiments involving these organisms might be desirable. She suggested that proposals involving nonpathogenic prokaryotes and nonpathogenic lower eukaryotes might be reviewed by ORDA rather than by the full RAC. ORDA could consult with experts in the field. Dr. Mason also expressed concern about the definition of nonpathogenicity, and supported the concept of ORDA review.

Dr. Gottesman moved a substitute motion to extend Section III-B-3 of the Guidelines, which currently allows recombinant DNA transfers between nonpathogenic prokaryotes at P3 containment, to include experiments with nonpathogenic lower eukaryotes. Her substitute motion would also

permit requests for lowering of containment for specific experiments in this class to be approved by ORDA. RAC review would not be required.

Dr. Mason seconded the substitute motion.

Dr. Brill said that the investigator would be most knowledgeable about the organism. He questioned whether ORDA review should be required.

Dr. Campbell spoke in support of Dr. Brill's motion. He felt the restrictions placed on recombinant DNA research were discouraging innovation.

Mr. Thornton called the vote on Dr. Gottesman's substitute motion. The RAC voted against Dr. Gottesman's motion by a vote of six in favor, eight opposed, and two abstentions.

Dr. Nightingale suggested that "nonpathogenic" is too absolute a term; certain organisms normally not pathogenic can cause disease in compromised hosts. Other members concurred.

Dr. Baltimore said the committee, in evaluating this proposal, should face the basic question of whether recombinant DNA technology is likely to produce an organism more pathogenic than the original donor organisms.

Dr. Levine said that while many members of the committee feel it is exceedingly unlikely that recombinant DNA technology will create a new pathogen of clinical significance, disparities could arise among institutions concerning whether a given organism is, or is not, a pathogen under Dr. Brill's proposal.

Mr. Thornton then called the vote on Dr. Brills' amended proposal. By a vote of nine in favor, eight opposed, and three abstentions, the RAC accepted Dr. Brill's proposal. Dr. Goldstein and Dr. Krinsky requested to be recorded as voting against the motion.

Dr. Gottesman, in noting the closeness of the vote, again offered for consideration her substitute proposal. Her proposal was that, if Dr. Fredrickson should not accept Dr. Brill's amended proposal, the sense of the RAC was that it would be preferable to the status quo to make at least two changes in the Guidelines: (1) to extend the current situation allowing cloning between nonpathogenic prokaryotes at P3 containment to include nonpathogenic lower eukaryotes, and (2) to allow lowering containment below P3 for individual cases in this class to be approved by ORDA, rather than requiring RAC review. She moved this proposal. Dr. Goldstein seconded. Dr. Berns moved to table discussion. Dr. Campbell seconded. By a vote of eight in favor, eleven opposed and no abstentions, the motion to table Dr. Gottesman's substitute motion failed.

Mr. Thornton then called the vote on Dr. Gottesman's motion. By a vote of fourteen in favor, one opposed, and three abstentions, the RAC approved Dr. Gottesman's motion.

VIII. CLOSED SESSION

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

IX. PROPOSALS TO CLONE GENES OF FOOT AND MOUTH DISEASE VIRUS

Dr. Gartland introduced a request (tab 972), dated October 17, 1980, from Genentech, Inc., and the United States Department of Agriculture Plum Island Animal Disease Center concerning the cloning of the Foot and Mouth Disease Virus (FMDV) genome. An earlier proposal entitled "Cloning and Expression in E. coli of the VP3 protein of Foot and Mouth Disease Virus" had been reviewed by the RAC at the December 6-7, 1979 meeting. On the recommendation of the RAC, Stage I of that proposal, the construction of clones containing cDNA segments of the FMDV genome, was approved by the NIH and announced in the Federal Register of January 17, 1980 (45 FR 3552). At that time it was noted that "Dr. Campbell stated that it was the sense of the RAC that this motion constituted the 'major action' and that future recommendations of the RAC approving further stages of the experiment would be 'minor actions.'" Subsequently, permission was given (Federal Register of July 29, 1980 (45 FR 50528)) that certain clones containing cDNA copies of the FMDV genome made on Plum Island could be removed from Plum Island as they "were well characterized, lacked infectivity, and represent, in aggregate, only 75% of the FMDV genome." Dr. Gartland said request 1 in the October 17, 1980, proposal dealing with work in E. coli K-12 could be considered a "minor action" continuation of the previously reviewed proposal. However, request 2 of the October 17, 1980, submission dealing with proposed work in hosts other than E. coli could not be so considered. Dr. Gartland suggested RAC might therefore appropriately evaluate request 1 (but not request 2) of the submission, even though the proposal (tab 972) had not

been published in the Federal Register for thirty days of public comment as the request had not been received by ORDA until December 1980.

Drs. Berns, Gottesman and Baltimore agreed that request 1 of the proposal could be appropriately considered at this meeting.

Dr. Baltimore, in reviewing request 1 of the proposal, explained that several types and subtypes of Foot and Mouth Disease Virus are endemic in the world today. He said vaccine producers must thus develop vaccines against multiple types and subtypes of the virus. He said Genentech, Inc., in the original proposal, had chosen one FMDV type as a prototype, requested and obtained NIH permission for experiments involving this type, and now is requesting permission to apply the same procedures to other FMDV serological types. Dr. Baltimore said Genentech, Inc., had agreed to abide by the conditions set by RAC. Dr. Campbell said it was appropriate that a RAC working group, but not the full RAC, review data on the infectivity of the clones before they are removed from Plum Island. Dr. Berns suggested that, as in the previous approval, the clones allowed to leave Plum Island shall not contain, individually or collectively, more than 75% of the viral genome. Dr. Baltimore included these statements in his motion to approve request 1 of the proposal. Dr. Fedoroff seconded the motion.

By a vote of 20 in favor, none opposed and one abstention, the RAC recommended approval of request 1 of the proposal (tab 972). This action would permit cloning on Plum Island of various FMDV types in E. coli K-12.

The following conditions were specified: (1) A working group of the RAC, but not the full RAC, would examine data on the infectivity of the clones produced on Plum Island before these clones were allowed to leave the Island, and (2) the clones to leave Plum Island should be well-characterized, shown to lack infectivity, and shall not contain, individually or collectively, more than 75% of the FMDV genome.

Dr. Gottesman suggested that some discussion of request 2 of the proposal (tab 972) was appropriate. Although no formal action should be taken at this meeting, a discussion might identify potential problems.

Dr. Baltimore said request 2 of the proposal (tab 972) asks permission for cloning of FMDV cDNA in Bacillus subtilis, Saccharomyces cerevisiae, and in eukaryotic cells in culture. He envisaged no potential hazard in cloning the VP3 protein in these host-vector systems. Dr. Ross of Genentech, Inc., said the VP3 protein has no known biological activity other than as a structural protein in the FMDV coat. Dr. Baltimore asked Dr. Ross if less than two-thirds of the SV40 genome would be used as a vector for cloning the VP3 protein. Dr. Ross replied that less than two-thirds of the SV40 genome would be used. Dr. Goldstein asked if inserting the gene for the VP3 protein into a two-third fragment of the SV40 genome could produce a viable virus with modified host range. Dr. Baltimore said that it is very unlikely that the VP3 protein could be inserted into the SV40 capsid structure.

Dr. Berns asked for a clarification of the proposal. He noted that the discussion focused on one of the FMDV capsid proteins, the VP3 protein. However, Genentech will have up to 75% of the entire viral genome, and the October 17, 1980 proposal requests permission to clone FMDV capsid proteins in general, not just VP3. Dr. Ross said that approval for just the VP3 protein would be acceptable at this time. Dr. Gottesman suggested that the proposal should be more explicit. It was also stated that additional information on the vectors to be used should be supplied.

The RAC deferred action on request 2 of tab 972.

X. PROPOSAL FOR APPROVAL OF SCHIZOSACCHAROMYCES POMBE FOR RECOMBINANT DNA EXPERIMENTS

Dr. Benjamin Hall of the University of Washington requested that the fission yeast, Schizosaccharomyces pombe, together with S. cerevisiae/E. coli hybrid recombinant plasmids, be certified as an HVI host-vector system (tabs 970, 975, 976/7). Dr. Hall in addition requested that this system be included in Section III-O of the Guidelines. Schizosaccharomyces pombe is nonpathogenic, survives poorly outside of controlled laboratory conditions, is rare in nature, and exchanges genetic information only with other closely related organisms. Dr. Campbell said the request is unusual in that the specified host is the species rather than a specific laboratory strain. He noted that the current HVI certified hosts are at a selective disadvantage relative to their wild type counterparts. He felt the RAC should adhere to criteria specified in the Guidelines for

certifying HVL host-vector systems. On this basis he said he could not recommend approval of the request. Dr. Gottesman agreed.

Dr. Pinon agreed, but suggested that the NIH might permit certain experiments with the organism, while not certifying Schizosaccharomyces pombe as an HVL host-vector system.

Dr. Campbell moved approval of the following motion:

"DNA from nonpathogenic prokaryotes and lower eukaryotes may be cloned into Schizosaccharomyces pombe species under P1 containment conditions."

By a vote of fourteen in favor, one opposed, and four abstentions RAC recommended the motion. Dr. Campbell then moved a second recommendation:

"DNA from higher eukaryotes may be cloned in Schizosaccharomyces pombe species under P3 containment conditions."

By a vote of fourteen in favor, none opposed, and five abstentions, RAC accepted the motion.

XI. REQUEST TO INCLUDE STREPTOCOCCUS FAECALIS AND STREPTOCOCCUS SANGUIS UNDER EXEMPTION I-E-4.

Dr. Gottesman introduced the request (tabs 967, 976/1) of Dr. Donald Clewell of the University of Michigan that Streptococcus faecalis be included along with Streptococcus sanguis in a sublist of Appendix A

of the Guidelines. Dr. Gottesman noted that Appendix A currently has two sublists, E and F, both of which include Streptococcus sanguis. She suggested that Streptococcus faecalis be added to sublist F if the RAC is satisfied that the evidence demonstrates exchange in both directions between S. faecalis and S. sanguis. Dr. Campbell supported that approach, and moved approval. Dr. Fedoroff seconded the motion.

By a vote of twelve in favor, two opposed, and five abstentions, the RAC recommended that the request be approved, and that Streptococcus faecalis be added to sublist F of Appendix A.

XII. PROPOSAL FOR CONTAINMENT FOR STREPTOMYCES AND NONPATHOGENIC ACTINOMYCETES

Dr. Levine introduced three requests (tab 974) of Dr. Stanley Cohen of Stanford University to revise containment levels for recombinant DNA experiments involving the nonpathogenic free-living soil organism genus Streptomyces and other nonpathogenic Actinomycetes. Dr. Talbot pointed out that this item had not been published in the Federal Register for thirty days of public comment due to its late receipt in ORDA. He noted however that the first of the three requests was a specific instance of a much broader proposal (see item VII of these minutes) which had been published in the Federal Register, and that, therefore, the RAC could appropriately act on this. Dr. Levine read the first of Dr. Cohen's requests:

"That all members of the nonpathogenic Actinomycetes genus Streptomyces and the plasmids native to this genus be approved as host-vector systems for the cloning under P1 conditions of DNA

derived from other non-pathogenic prokaryotic organisms such as Streptomyces and other non-pathogenic Actinomycetes species, Escherichia coli K-12, Bacillus subtilis, Bacillus lichenformis, Bacillus circulans, and other non-pathogenic Bacillus species, and for the cloning of DNA derived from non-pathogenic unicellular eukaryotic microorganisms such as Saccharomyces cerevisiae and Neurospora crassa."

Dr. Levine noted that the RAC had earlier in the meeting (see item VII of these minutes) recommended P1 containment as sufficient for a large class of experiments, of which these were a small subset. He recommended approval. Dr. Maas seconded the motion.

By a vote of eighteen in favor, none opposed, and two abstentions, the RAC recommended approval of the first request of tab 974. Dr. Campbell abstained from discussing and voting on this request.

The RAC deferred action on the second and third requests of tab 974.

Dr. Gottesman said that Dr. Cohen should supply additional data concerning the organisms he wishes to be certified as HVI host-vector systems.

Dr. Williams concurred.

XIII. PROPOSAL TO AMEND ITEM 4 OF APPENDIX E

Dr. Brill introduced the proposal (tabs 968, 976/5) by Dr. Clarence Kado of the University of California, Davis, to modify item 4 of Appendix E.

Dr. Kado requested deletion of the specification that experiments involving Agrobacterium tumefaciens be performed "under containment conditions" one

step higher than would be required for the desired DNA in HVI systems (i.e., one step higher physical containment than that specified in the subsections of Section III-A)."

According to Dr. Kado's proposal, item 4 of Appendix E would read as follows:

"Cloned desired fragments from any non-prohibited source may be transferred into Agrobacterium tumefaciens containing a Ti plasmid (or derivatives thereof), using a nonconjugative E. coli plasmid vector coupled to a fragment of the Ti plasmid and/or the origin of replication of an Agrobacterium plasmid, under containment conditions that would be required for the desired DNA in HVI systems (i.e., that specified in the subsections of Section III-A). Transfer into plant parts or cells in culture would be permitted at the same containment level."

In support of the request, Dr. Brill said that Agrobacterium tumefaciens, while a pathogen, does not attack tissue which has not been injured. Expression of the Ti plasmid coded genes is diluted out as the plant grows. Furthermore, the Ti plasmid is apparently lost in meiotic segregation, and is not transmitted to progeny. He moved approval of Dr. Kado's proposal. Dr. Scandalios concurred.

Dr. Gottesman noted that the proposal would effectively designate Agrobacterium tumefaciens an HVI system. She did not find this action appropriate as (1) the Agrobacterium tumefaciens Ti plasmid is a conjugative plasmid, and (2) although Agrobacterium tumefaciens is not a serious

pathogen, it is, nonetheless, a pathogen. She suggested that containment conditions for certain types of experiments might appropriately be relaxed, but suggested that the RAC refrain from designating Agrobacterium tumefaciens a de facto HVI system. She proposed to lower containment to P2 for experiments involving cloning DNA from non-pathogenic prokaryotes and plants in Agrobacterium tumefaciens with subsequent transfer to plants or plant tissue. Dr. Brill accepted this proposed amendment of his motion.

The motion was recommended by the RAC by a vote of seventeen in favor, none opposed, and two abstentions.

XIV. REQUEST FOR LOWERING OF CONTAINMENT UNDER ITEM 4 OF APPENDIX E

Dr. Scandalios introduced the request (tabs 969, 976/6) of Dr. Mary-Dell Chilton of Washington University in St. Louis to reduce physical containment to P2 for the manipulation in Agrobacterium tumefaciens of

- (1) the Saccharomyces cerevisiae alcohol dehydrogenase 1 gene and
- (2) the gene coding for the maize (Zea mays) seed storage protein, zein.

The cloned DNA and the vectors will be introduced into tobacco plants. Dr. Scandalios said these experiments are currently covered by item 4 of Appendix E which specifies P3 containment conditions. He said Dr. Chilton requests a lowering of containment as the recombinant DNAs used in the manipulations are well-characterized. Dr. Scandalios recommended that the specified experiments be permitted under P1 containment conditions and so moved. Dr. Brill concurred.

By a vote of fifteen in favor, none opposed, and four abstentions, the RAC recommended Dr. Scandalios' motion.

XV. REQUEST TO CLONE SACCHAROMYCES CEREVISIAE DNA IN TETRAHYMENA

Dr. Maas began discussion of the request (tabs 979, 976/4) of Dr. Eduardo Orias of the University of California, Santa Barbara, to clone Saccharomyces cerevisiae DNA in Tetrahymena thermophila using S. cerevisiae/E. coli hybrid plasmids. Dr. Orias, in support of his request, noted that Tetrahymena thermophila is a unicellular eukaryote of no known pathogenicity. Dissemination of genetic information between members of the species by means other than eukaryotic conjugation has not been demonstrated.

Dr. Levine said this request is an example of experiments which would be covered by the new proposed mechanism concerning non-pathogenic prokaryotes and non-pathogenic lower eukaryotes (see item VII of these minutes).

Dr. Maas moved acceptance of the proposal at the P1 containment level.

Dr. Levine seconded the motion. By a unanimous vote of nineteen in favor, RAC recommended the action.

XVI. DRAFT PROPOSAL ON TOXINS

Dr. Maas said, in his mind, the cloning of toxin genes may be among the few real potential hazards posed by recombinant DNA experiments.

Dr. Maas said an ad hoc group composed of Dr. Alan Bernheimer of New York University, Dr. John Collier of Yale University, Dr. Michael Gill of Tufts University, Dr. Susan Gottesman of NIH, Dr. Myron Levine of

the University of Maryland, Dr. James Mason of the Utah State Department of Health, and himself had met to consider appropriate containment for recombinant DNA experiments involving genes coding for toxins.

Dr. Maas said the group had participated in two telephone conference calls and had met on January 7, 1981 to develop language for publication in the Federal Register and for subsequent consideration at the April 23-24, 1981 RAC meeting. He presented the draft language developed at the January 7, 1981 meeting (Attachment II).

Dr. Maas said the proposal could be broken down into three parts: (1) a preamble which offers the logic and reasoning behind the proposal, (2) a section outlining procedures to determine toxicity and (3) containment conditions for various potencies. He noted that for the moment the proposal dealt only with cloning in E. coli K-12 host-vector systems.

Dr. Maas suggested that the ad hoc working group might be consulted when proposals evaluating toxins are evaluated.

Mr. Thornton suggested that each RAC member take the opportunity to make suggestions on the draft proposal. He thanked the ad hoc working group for their efforts. Dr. Berns suggested that Dr. Gill might be invited as an ad hoc consultant to the RAC meeting at which the toxin proposal will be considered.

XVII. FUTURE MEETING DATES

Mr. Thornton directed the attention of the members to tab 961 which outlined future meeting dates of the RAC. These are April 23 and 24, 1981, September 10 and 11, 1981, and January 7 and 8, 1982.

XVIII. ADJOURNMENT

The meeting was adjourned at 12:15 p.m., January 9, 1981.

Respectively submitted,

Elizabeth A. Milewski, Ph.D.
Rapporteur

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

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

Date

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

RECOMBINANT DNA ADVISORY COMMITTEE

CHAIRMAN

THORNTON, Ray, J.D. (82)
 President
 Arkansas State University
 State University, Arkansas 72467
 501 972-2100

<p>AHMED, Abdul Karim, Ph.D. (82) Senior Staff Scientist Natural Resources Defense Council, Inc. 122 East 42nd Street New York, New York 10017 212 949-0049</p>	<p>CASON, Zelma (81) Supervisor Cytopathology Laboratory Department of Pathology University of Mississippi Medical Center Jackson, Mississippi 39216 601 987-5547</p>
<p>BALTIMORE, David, Ph.D. (82) Professor of Biology Center for Cancer Research Massachusetts Institute of Technology Cambridge, Massachusetts 02139 617 253-6410</p>	<p>FEDOROFF, Nina V., Ph.D. (84) Staff Member Department of Embryology Carnegie Institution of Washington 115 West University Parkway Baltimore, Maryland 21210 301 467-1414</p>
<p>BERNS, Kenneth I., Ph.D., M.D. (83) Chairman Department of Immunology and Medical Microbiology University of Florida College of Medicine Gainesville, Florida 32610 904 392-3311</p>	<p>GOLDSTEIN, Richard, Ph.D. (82) Associate Professor Department of Microbiology and Molecular Genetics Harvard Medical School Boston, Massachusetts 02115 617 732-1911</p>
<p>BRILL, Winston J., Ph.D. (83) Vilas Research Professor Department of Bacteriology University of Wisconsin Madison, Wisconsin 53706 608 262-3567</p>	<p>GOTTESMAN, Susan K., Ph.D. (81) Senior Investigator Laboratory of Molecular Biology National Cancer Institute National Institutes of Health Bethesda, Maryland 20205 301 496-2095</p>
<p>CAMPBELL, Allan M., Ph.D. (81) Professor Department of Biological Sciences Stanford University Stanford, California 94305 415 497-1170</p>	<p>HARRIS, Jean L., M.D. (83) Secretary of Human Resources Commonwealth of Virginia Office of Governor Post Office Box 1475 Richmond, Virginia 23212 804 786-7765</p>

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HOLMES, King K., M.D., Ph.D. (84) Head Division of Infectious Diseases U.S. Public Health Service Hospital Seattle, Washington 98114 206 325-2997	McGARRITY, Gerard J., Ph.D. (84) Head Department of Microbiology Institute for Medical Research Copewood Street Camden, New Jersey 08103 609 966-7377
KING, Patricia A., J.D. (82) Deputy Assistant Attorney General Civil Division Department of Justice 10th & Constitution Ave., N.W. Washington, D.C. 20530 202 724-6841	McKINNEY, Robert W., Ph.D. (84) Chief, Occupational Safety and Health Branch Division of Safety National Institutes of Health Bethesda, Maryland 20205 301 496-2960
KRIMSKY, Sheldon, Ph.D. (81) Acting Director Program in Urban Social and Environmental Policy Tufts University Medford, Massachusetts 02155 617 628-5000 x726	NIGHTINGALE, Elena O., Ph.D., M.D. (83) Senior Program Officer Institute of Medicine National Academy of Sciences Washington, D.C. 20418 202 389-6721
LEVINE, Myron M., M.D. (84) Director Center for Vaccine Development Division of Infectious Diseases University of Maryland School of Medicine Baltimore, Maryland 21201 301 528-7588	NOVICK, Richard P., M.D. (81) Member and Chief Department of Plasmid Biology Public Health Research Institute of the City of New York, Inc. New York, New York 10016 212 481-0746
MAAS, Werner K., Ph.D. (83) Professor Department of Microbiology New York University School of Medicine New York, New York 10016 212 340-5322	PARKINSON, David K., B.M., B.Ch. (81) Associate Professor Graduate School of Public Health University of Pittsburgh Pittsburgh, Pennsylvania 15261 412 624-3041
MASON, James O., M.D., Dr. Ph. (83) Executive Director Utah State Department of Health Post Office Box 2500 Salt Lake City, Utah 84110 801 533-6111	PINON, Ramon, Ph.D. (82) Associate Professor Department of Biology B-022 Bonner Hall University of California, San Diego La Jolla, California 92093 714 452-2452

- 3 -

SCANDALIOS, John G., Ph.D. (84)
Head
Department of Genetics
North Carolina State University
Raleigh, North Carolina 27650
919 737-2291

WILLIAMS, Luther S., Ph.D. (81)
Professor
Department of Biology
Washington University
Campus Box 1137
St. Louis, Missouri 63130
314 889-6843

EXECUTIVE SECRETARY

GARTLAND, William J., Jr., Ph.D.
Director, Office of Recombinant
DNA Activities
National Institute of Allergy
and Infectious Diseases
National Institutes of Health
Bethesda, Maryland 20205
301 496-6051

RECOMBINANT DNA ADVISORY COMMITTEE

NON-VOTING REPRESENTATIVES

CENTERS FOR DISEASE CONTROL

DOWDLE, Walter R., Ph.D.
 Assistant Director for Science
 Centers for Disease Control
 Atlanta, Georgia 30333
 404 329-3701

National Institute for Occupational Safety and Health (CDC)

LEMIN, Richard A.
 Director
 Office of Program Planning
 and Evaluation
 National Institute for Occupational
 Safety and Health,
 Room 8A53
 5600 Fishers Lane
 Rockville, Maryland 20857
 301 443-3680

U.S. DEPARTMENT OF AGRICULTURE

TOLIN, Sue A., Ph.D.
 Science and Education Administration
 Cooperative Research
 U.S. Department of Agriculture
 Washington, D.C. 20250
 202 447-5741

FULKERSON, John F., Ph.D. (ALT)
 Science and Education Administration
 Cooperative Research
 U.S. Department of Agriculture
 Washington, D.C. 20250
 202 447-5741

U.S. DEPARTMENT OF COMMERCE

GORDON, George S., Ph.D.
 Analyst
 Office of Environmental Affairs
 U.S. Department of Commerce
 Room 3425
 Washington, D.C. 20230
 202 377-2565

PAYNTER, O. E., Ph.D. (ALT)
 Toxicologist
 Office of Environmental Affairs
 U.S. Department of Commerce
 Room 3425
 Washington, D.C. 20230
 202 377-3234

U.S. DEPARTMENT OF ENERGY

DUDA, George, Ph.D.
 Office of Health and
 Environmental Research, EV-33
 U.S. Department of Energy
 Washington, D.C. 20545
 202 353-3651

EDINGTON, Charles W., Ph.D. (ALT)
 Deputy Director
 Office of Health and Environmental
 Research
 U.S. Department of Energy
 Washington, D.C. 20250
 202 353-3251

- 2 -

U.S. DEPARTMENT OF THE INTERIOR

PIMENTEL, Mariano B., Ph.D.
Medical Director
U.S. Department of the Interior
Room 7045
18th & C Street, N.W.
Washington, D.C. 20240
202 343-2081

U.S. DEPARTMENT OF JUSTICE

BELL, Charlotte R., J.D.
General Litigation Section
Land and Natural Resources
Division
U.S. Department of Justice
Washington, D.C. 20530
202 633-4150

U.S. DEPARTMENT OF STATE

WALSH, William J., III
Biomedical Research Liason
and Health Affairs Officer
Oceans and International Environmental
and Scientific Affairs
U.S. Department of State
Washington, D.C. 20520
202 632-4824

U.S. DEPARTMENT OF TRANSPORTATION

CUSHNAC, George E., Ph.D.
Chemist
Research and Special Programs
Administration
U.S. Department of Transportation
Washington, D.C. 20590
202 755-4906

- 3 -

U.S. ENVIRONMENTAL PROTECTION AGENCY

BANKS, Darryl R., Ph.D.
Executive Assistant to the
Assistant Administrator for
Research and Development
Office of Research and Development
United States Environmental Protection Agency
RD 672, 913 West Tower
Washington, D.C. 20460
202 755-0122

FOOD AND DRUG ADMINISTRATION

HENRY, Timothy J., Ph.D.
BF, DT, HFF-156
Food and Drug Administration
200 C Street, S.W.
Washington, D.C. 20204
202 472-4690

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

DeVINCENZI, Donald L., Ph.D.
Program Manager, Planetary Biology
Code SBL-3
National Aeronautics and Space
Administration
Washington, D.C. 20546
202 755-3732

NATIONAL SCIENCE FOUNDATION

LEWIS, Herman W., Ph.D.
Senior Scientist for Recombinant DNA
Division of Physiology
Cellular and Molecular Biology
National Science Foundation
Washington, D.C. 20550
202 357-7647

HARRIMAN, Phillip, Ph.D. (ALT)
Program Director for Genetic Biology
Room 326
National Science Foundation
Washington, D.C. 20550
202 632-5985

- 4 -

U.S. VETERANS ADMINISTRATION

SCHULTZ, Jane S., Ph.D.
Geneticist
U.S. Veterans Administration
Medical Research Service - 151
2215 Fuller Road
Ann Arbor, Michigan 48105
313 769-7100 x696

BERMAN, Howard M. (ALT)
Health Scientist
Program Development and Review
Division
U.S. Veterans Administration
810 Vermont Avenue, N.W.
Washington, D.C. 20420
202 389-5065

U.S. DEPARTMENT OF LABOR

LOGAN, David C., M.D.
Medical Officer
Office of Technical Support
Occupational Safety and Health
Room N3656
U.S. Department of Labor
Washington, D.C. 20210
202 523-9603

RECOMBINANT DNA ADVISORY COMMITTEE

LIAISON REPRESENTATIVES

JUENGST, Eric T. (Acting)
Program Specialist
Program of Science Technology
& Human Value
Mail Stop 104
National Endowment for the Humanities
Washington, D.C. 20506
202 474-0354

WEISS, Daniel L., M.D.
Assembly of Life Sciences
National Academy of Sciences
Washington, D.C. 20418
202 389-6315

IINO, Professor Tetsuo
Faculty Science
University of Tokyo
Hongo, Tokyo 113
Japan

FIRST DRAFT PRESENT TO RAC ON JANUARY 9, 1981

Cloning of Toxins

I. Preamble

Whereas it is unlikely that novel pathogens of clinical significance for man might be created by the cloning of genes for toxic proteins into new host bacteria that colonize humans or that may pass genetic information to organisms capable of colonizing humans, it is nevertheless prudent to restrict the cloning of genes for potent toxins.

The theoretical dangers stem from the habits of the new bacterial host and the toxicity of the toxin per se rather than known attributes of the organism that contributes the toxin gene(s), its ecology, virulence, amount of toxin it may synthesize in humans or elsewhere, and the possibility that it exchanges genetic information with certain other organisms in nature. Likewise, the toxins role, or otherwise, in pathogenicity of the donor organism is not necessarily of relevance.

The extent to which toxins are a danger is usually difficult to ascertain for humans. The specification thus attempts to define a level of activity below which proteins might be considered safe and specifies minimal acceptable safety tests on animals which might predict human safety levels. Because there are wide ($>10^5$ fold) differences in susceptibilities of animals to toxins, human safety may be inferred with reasonable assurance only if an agent is shown to non-potent to another primate or to several lower mammals. The specification is worded so that non-potency (potency) for lower animals would be over-ridden by evidence of potency (non-potency) to primates or humans.

DRAFT 1/7/91

Additional precautions may be desirable if synergy or potentiation occur as, for example those bacterial exotoxins that enhance the toxicity of endotoxin.

Toxicity Determination

A toxin shall be considered potent at a certain level if parenteral administration of a certain amount causes death, disfigurement or profound neurological effects.

- a) If the human toxicity is known, this information shall be paramount.
- b) If human toxicity is not known, it may be inferred pro tem from assays of toxicity to another primate (intravenous injection to at least four animals).
- c) If neither human nor other primate toxicity is known, human toxicity may be inferred from 16 most sensitive of three small animals, namely mice, guinea pigs and rabbits, using intravenous injection into at least four animals of each species.

The toxin used for the tests must be of good quality without substantial denaturation or chemical alteration from its most effective form. The purity must be known sufficiently to determine the content of specific agent. If the purity is in doubt the most conservative assumption must be made. An impure toxin that appears similar in structure and action to a known toxin may be assumed pro tem to be ten times as toxic as the known toxin.

When two or more proteins act in synergy to form a toxic principle and the components are to be cloned separately under conditions that rigorously preclude the comingling of the separate clones, the toxicities of each component may be considered individually.

Restrictions

1. No specific restriction shall apply to the cloning of DNA specifying a protein if it is non-potent when administered at the level of 100 μ g (or more) per kilogram of body weight.
2. Cloning of Class A, B and C toxins is, for the present, restricted to E. coli host-vector systems. Class A toxins are defined as those that are potent at the level of 1 μ g - 100 μ g/kg body weight. These may be cloned in EK1 Pl.

Special Case:

Some enterotoxins are substantially more toxic when administered enterally than parenterally and must be considered separately. The following enterotoxins whose effects are confined to the stimulation of intestinal secretion that can be entirely reversed by administration of electrolyte solutions shall be subject to the rules governing Class A toxins. The heat stable toxins of E. coli (both STI & STII) and of Y. enterocolitica cholera toxin, the heat labile toxins of E. coli, Klebsiella, and other related proteins as may be identified that are neutralized by an antiserum monospecific for cholera toxin.

3. Class B toxins are those that are potent at the level of 100 ng - 1000 ng/kg body weight (examples - diphtheria toxin, Clostridium perfringens, epsilon toxin, abrin). DNA for these proteins may be cloned EK2/P2 or EK1/P3.
4. Class C toxins are potent at less than 100 ng, namely the botulinum toxins, tetanus toxin, and Shigella dysenteriae neurotoxin. Cloning of genes for these toxins is restricted but exceptions will be considered on a case-by-case basis by RAC following publication of the request in the Federal Register. It is likely that permission to proceed with some protocols involving toxins of this class will require the work to proceed in a P4 facility.

Footnote:

It is conceivable that some toxins may greatly (≥ 100 -fold) potentiate the effects of other toxins. If information on potentiation becomes available, the toxins with potentiating effects on other toxins will constitute a special situation to be considered on a case-by-case basis as a minor action.

LD₅₀ or MLD/kg1-10 ng

	Man	Monkey	Mouse	Guinea Pig	Rabbit
Botulinum toxin A	10 ng		1.2 ng	(0.6 ng)	(0.5 ng)
B			1.2 ng	0.6 ng	
C		1/3 mouse		1 x mouse	1/8 mouse
D		(40 ng)	<0.4 ng	(0.1 ng)	(0.08 ng)
E proteolytically activated		(1.1 ng)	1.1 ng	(0.6 ng)	(1.1 ng)
F					
Tetanus toxin	<2.5 ng		7 ng	~2 ng	(.05-5 ng)
Shigella dysenteriae neurotoxin		(4.5 ng)	1.3 µg	>9 µg	0.9 ng

10-100 ng100-1000 ng

Diphtheria toxin	≤100 ng		200 µg	160 ng	
Abrin			600 ng		
Cl. perfringens Epsilon toxin (trypsin activated)			250 ng 1 µg		

1-10 µg

Staphylococcal Alpha toxin			40-60 µg		1.3 µg
Ricin			3 µg		
Pseudomonas aeruginosa exotoxin A			3 µg		
Streptolysin O			10-25 µg	as rabbit	3 µg
Cl. perfringens Theta toxin			13-16 µg		5-8 µg
Pneumolysin		O-labile hemolysins			4.4 µg
Cereolysin			40-80 µg		
Listeriolysin			3-12 µg		

and presumably likewise for similar hemolysins produced by other Clostridium and Bacillus species

10-100 µg

P. pestis murine toxins A or B			35 µg, ~50 µg		
B. pertussis toxin			< or << 60 µg, 5 µg		
B. anthracis. Lethal factor (with PA)			(rat) <114 µg		
S. aureus Beta toxin			500 µg-5mg	40-400 µg	3-30 µg ?

	Man	Monkey	Mouse	Guinea Pig	Rabbit
100 ug- 1, mg					
Shiga Toxin (i.v.)			250 µg (less enterally)		
ST (i.v.)			presumed 250 µg		
St. perfringens enterotoxin			300 µg		
Dord Factor			500 µg		
1-10 mg					
Pseudomonas aeruginosa protease(s)			≥ 3mg, 4 mg		
Streptococcal erythrogenic toxin (also enhances effect of endotoxin)			3.6 mg		3.5 mg
Proteus mirabilis neurotoxin S			3 mg		
10-100 mg					
Staphylococcal Gamma toxin			< 50 mg ?		
Staphylococcal Delta toxin					40 mg ?
S. aureus enterotoxin					
A	} po	~ 20 ng	~ 2 µg		
B		< 500 ng	~ 2 µg		
C					
A	} iv				
B			0.1 ug		
C					
Staph leukocidin					> 1 mg
Toxic shock toxin					
Legionella					
B. cereus enterotoxins					
ST					
C. difficile					
Streptolysin S					