

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

RECOMBINANT DNA MOLECULE PROGRAM ADVISORY COMMITTEE

MINUTES OF MEETING<sup>1</sup>

APRIL 27-28, 1978

The Recombinant DNA Molecule Program Advisory Committee (RAC) was convened for its eleventh meeting at 9 a.m. on April 27, 1978, in Conference Room 10, Building 31C, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland. Dr. DeWitt Stetten, Jr., (Chairman) Deputy Director for Science presided. In accordance with Public Law 92-463 the meeting was open to the public.

Committee members present were:

Drs. Edward A. Adelberg; Allan M. Campbell; Peter R. Day; Susan K. Gottesman; Donald R. Helinski; Richard B. Hornick; Elizabeth M. Kutter; Emmette S. Redford; Wallace P. Rowe; Jane K. Setlow; John Spizizen; LeRoy Walters; Milton Zaitlin; and William J. Gartland, Jr., Executive Secretary.

A Committee roster is attached. (Attachment I)

The following ad hoc consultants to the Committee were present:

Dr. Stanley Falkow, University of Washington.  
Dr. Harold Ginsberg, Columbia College of Physicians and Surgeons, on sabbatical at Rockefeller University.

Other National Institutes of Health staff present were:

Dr. Stanley Barban, NIAID; Dr. Emmett Barkley, NCI; Dr. Fred Bergmann, NIGMS; Mr. George Boden, OD; Mrs. Betty Butler, NIGMS; Dr. Philip Chen, OD; Dr. Irving Delappe, NIAID; Mr. Tom Flavin, OD; Dr. Donald Fredrickson, Director; Dr. Michael Goldberg, NIGMS; Mrs. Florence Hassell, OD; Mr. Joe Hernandez, OD; Dr. John Irwin, DRS; Dr. Daphne Kamely, NIGMS; Dr. Ruth Kirschstein, NIGMS; Dr. Malcolm Martin, NIAID; Dr. John Norvell, NIGMS; Dr. John Nutter, NIAID; Dr. Joseph Perpich, OD; Dr. Bernard Talbot, OD; Dr. Rudolf Wanner, DRS.

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

The following liaison representatives were present:

Dr. George Duda, Department of Energy; Dr. Richard Hedrich, National Endowment for the Humanities; Dr. Herman Lewis, National Science Foundation; Dr. Daniel Weiss, National Academy of Sciences.

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

Dr. Giorgio Bernardi, National Research Center, Paris; Dr. John Baxter, University of California, San Francisco; Dr. Naum Bers, Rockville, Maryland; Dr. Paul Burnett, Eli Lilly Research Laboratories; Mr. Rick Curtin, JRB Associates; Dr. T. A. Fraser, Upjohn Research Laboratories; Dr. Denise Friello, GE Research and Development; Ms. Edith Godette, OSHA; Mr. Sandy Grimwade, Nature; Mr. Robert M. Hering, The Blue Sheet; Dr. George E. Holmes, Howard University; Dr. Paul Hung, Abbott Laboratories; Dr. Marilyn Hutchinson, NIOSH; Dr. R. P. Kahn, APHIS, USDA; Dr. P. J. Laipis, University of Florida; Dr. Louis LaMotte, Jr., Center for Disease Control; Dr. Paul Lovette, University of Maryland; Ms. Kathy Majerus, OSHA; Dr. James McCullough, Library of Congress; Mr. Richard Riseberg, Office of the General Counsel, HEW; Dr. Wacław Szybalski, University of Wisconsin; Mr. G. Tenenbaum, Brandeis University; Dr. Charles Weiner, Massachusetts Institute of Technology; Dr. Susan Wright, Ann Arbor, Michigan; Dr. R. S. Young, NASA.

I. CALL TO ORDER AND OPENING REMARKS

Dr. Stetten called the meeting to order at 9 a.m., April 27. He introduced Dr. Donald Fredrickson, Director, NIH. Dr. Fredrickson summarized the activities at NIH since the current version of the Guidelines for Recombinant DNA Research was issued in June 1976. He stated that he was convinced that through the process used to release the Guidelines and publication of the Environmental Impact Statement the public had considerable input into the development of standards for recombinant DNA research. He also stated that specialists in a variety of disciplines had carefully scrutinized the premises on which the Guidelines were based.

Dr. Fredrickson went on to list the conclusions that had been drawn concerning recombinant DNA research. There is no evidence to date that manipulations of recombinant DNA molecules yield any harmful products. Additionally, there is no evidence, since the publication of the Guidelines in 1976, to indicate that the probability of harm is likely. The results of the Falmouth conference on Risk Assessment of Recombinant DNA Experimentation with *Escherichia coli* K-12 [Executive Secretary's Note: See Journal of Infectious Diseases 137, 609-714 [1978]] indicate that *E. coli* K-12 cannot be converted to an epidemic pathogen. Scientists attending the U.S. - EMBO Workshop to Assess Risks for Recombinant DNA Experiments Involving the Genomes of Animal, Plant, and Insect Viruses (Federal Register, 43, 13748 (1978)) concluded that viral genomes or fragments thereof cloned in *E. coli* K-12 using approved plasmid or phage vectors

pose no more risk than work with the infectious virus or its nucleic acid and in most, if not all, cases clearly present less risk. Similar conclusions have been reached by agricultural scientists, meeting under the auspices of the USDA, NSF, and NIH, who concluded that current containment levels for the cloning of DNA from plant pathogens in E. coli K-12 are too high. Dr. Fredrickson stated that, although it was not possible to reduce the risk to zero, the burden of proof should now shift to the opponents of the research. Dr. Fredrickson then summarized the recent steps taken by the NIH. At the meeting of the Director's Advisory Committee in December 1977, there was universal sentiment for the exemption of certain experiments from prohibition on the basis of a need for risk assessment. There was also agreement on the need for exemptions for most self-cloning experiments, and for experiments involving organisms that are known to exchange genetic material.

Dr. Fredrickson noted that Guidelines in other countries placed fewer restrictions on scientists. The current Guidelines are too complex and must be revised on the basis of new evidence prior to their possible promulgation as regulations. He then briefly described the procedures associated with release of a revised version of the Guidelines. This will include publication of the proposed revised Guidelines together with an Environmental Impact Assessment and a decision document explaining the rationale for the revisions. The public would have a period of time to comment on the proposed revised Guidelines. A final version would then be published.

Dr. Fredrickson noted that members of the DAC and public commentators had suggested further revisions of the Guidelines. The RAC at this meeting must take these suggestions into consideration and make further recommendations. Among the issues that need to be addressed are a revised definition of recombinant DNA, changes in the scope of the Guidelines, a list of exempted exchangers, alterations in the roles and responsibilities of the investigator, institution and NIH, and the changes in the containment guidelines for covered experiments particularly those involving viruses and plant pathogens. Dr. Fredrickson continued by describing in further detail the scope of some of the changes. He noted that in the proposed revision the list of prohibited experiments would precede any list of exemptions. There was no need at the moment to remove any prohibitions. The following five classes of exemptions are proposed: recombinant DNAs not in organisms or viruses, recombinant DNAs from a single non-chromosomal source, recombinant DNAs from a host when propagated in that host, recombinant DNAs from species that exchange DNA by physiological processes, and recombinant DNAs that do not present a significant risk to health or the environment. In response to a question from Dr. Helinski of the RAC, Dr. Fredrickson stated that the definition of exchanging species could include both chromosomal and plasmid exchangers as long as standards were set. He also pointed out that the definition of recombinant DNA did not represent a change from a previous draft, but now included the concepts of exemptions and synthetic DNA.

Dr. Redford asked whether adequate public notice has been given for the proposed revisions of the virus and plant sections of the Guidelines. Dr. Fredrickson noted that a report of the U.S. - EMBO meeting had been published in the Federal Register. The proceedings of the Falmouth meeting will be published in the Journal of Infectious Diseases. In addition, the Minutes of this meeting will also be printed and made available to the public. Dr. Fredrickson indicated that in its review the RAC may recommend alterations of specific sections in the Guidelines.

Dr. Fredrickson described other changes in the roles and responsibilities section of the Guidelines. The revised Guidelines would extend to all recombinant DNA research at institutions receiving NIH funds for recombinant DNA research. He indicated that more responsibility for compliance and monitoring would be delegated to the local level. All Chairmen of IBC's will be invited to attend an NIH-sponsored briefing at which these new responsibilities would be explained. The shift in the locus of responsibility would include the following conditions: an explicit single set of standards for the conduct of the research, necessary competence within the IBCs, a clear understanding that sanctions would be imposed for non-compliance, post-hoc review of all IBC actions by ORDA, and mandated public participation on the IBCs. Dr. Fredrickson also stated that there would be a compliance section with penalties for violation of the Guidelines that could extend to cessation of all grant support.

Dr. Fredrickson then discussed the need to permit voluntary registration of non-NIH recombinant DNA projects, particularly in the absence of legislation. He asked the RAC to consider the question and decide upon their willingness to review industrial projects. He noted that they must keep in mind penalties for the unauthorized release of proprietary information. Dr. Kutter asked about IBC responsibilities in the event of non-compliance, particularly where there were repeated violations. Dr. Fredrickson replied that these situations will have to be considered on a case-by-case basis. Dr. Redford asked how it would be possible to check on the activities of IBCs. Dr. Fredrickson indicated that there will be no NIH inspection force and that legislation, if passed, would give such responsibility to another agency. NIH will monitor membership of IBCs and aid in self-regulation. A related issue was raised by Dr. Susan Wright who expressed concern that the shift of approval authority to the IBC was a major change in the review procedure. She asked whether there would be a further public hearing on this proposal. Dr. Fredrickson replied that a public hearing was under consideration (note added after meeting: a public meeting under the auspices of the Secretary will be held September 15, 1978). In response to a question about interagency cooperation, Dr. Fredrickson replied that these changes only applied to NIH, but he hoped that other agencies would accept them as well.

At the conclusion of the discussion Dr. Fredrickson noted that this would be Dr. Stetten's last meeting as Chairman of the RAC, and complimented him for his service in that capacity since the first meeting of the RAC in February 1975.

II. SUMMARY OF RECOMMENDATIONS FOR CHANGES IN THE PROPOSED REVISED GUIDELINES - SELECTED ISSUES FOR COMMITTEE REVIEW

Attached to the Minutes (Attachment II) is a document entitled "Selected Issues for Committee Review" that was sent to RAC members. A number of questions were addressed to the RAC. For the purpose of the Minutes Attachment II has been annotated in the margin to indicate questions, i.e., A, B, C, etc.

- A. The RAC unanimously accepted this suggestion that the definition of recombinant DNA (and therefore the scope of the Guidelines) cover recombinant DNA molecules whether inside or outside of organisms. The "prohibitions" and "exemptions" sections to follow will then make recombinant DNA molecules outside of organisms or viruses either prohibited (for example, derived from Class 3, 4, or 5 organisms as defined in the CDC "Classification of Etiologic Agents on the Basis of Hazard") or exempt from the Guidelines (see below).
- B. The RAC accepted the suggestion that the definition of recombinant DNA not include the items "capacity to infect" or "natural physiological processes." The proposed substitute definition in Attachment II was accepted, except that the RAC wanted the words "or be integrated into the genome of" to be deleted. Their suggested definition would thus speak to "molecules that have been constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell."
- C. The RAC concurred with the concept of moving the prohibited experiments before the exempted experiments so that the exemptions would not apply to experiments previously described as being prohibited. The Committee then entered into a lengthy and detailed discussion of the five classes of exempted experiments (see Attachment III for proposed list of exemptions).

Class i:

The RAC adopted exemption (i), but then voted (10 in favor, 2 opposed) to delete all material from exemption (i) which appeared in parentheses and move it instead to Appendix D of

the proposed revised Guidelines. Dr. Stetten expressed the reasoning for this as that one should not exempt something from guidelines and then immediately turn around and give guidance. This matter was brought up again later, with the suggestion that only part of this parenthesis be deleted but that there be retained the part which says "(However these should be inactivated prior to disposal)." This motion was defeated 7 votes to 6.

A motion then passed unanimously to add at the end of exemption (i), "(See Appendix D)."

Class ii:

The RAC unanimously adopted exemption (ii) of Attachment II.

Class iii:

The RAC adopted exemption (iii), but then rewrote it as follows:

- "iii. Those that are derived from plasmids or viruses indigenous to a bacterial host (or found in nature in that host) where the recombinant DNA molecules prepared from those plasmids or viruses and host genome are propagated within the host (or a closely related member of the same species)."

Following this, a motion to strike the word "bacterial" in iii was defeated 8 votes to 5.

On the next day, a second part was added to exemption iii (modified slightly after the RAC meeting by Drs. Zaitlin and Talbot for clarity)-namely, "or derived entirely from the organelles (e.g., chloroplasts or mitochondria) of an organism where the recombinant DNA molecules prepared from the organelles are propagated within that organism (or a closely related member of the same species)."

Class iv:

The RAC adopted exemption (iv) with the word "chromosomal" deleted.

On the first day of the meeting, Dr. Falkow presented to the RAC a series of possible lists of organisms which could be recommended by the RAC to form the initial list of "species that exchange DNA" as discussed in exemption iv. Three different lists were discussed in

detail. Votes were taken on acceptance of the lists or of the principles used to construct the lists. When Dr. Falkow presented his lists on the first day of the meeting, adoption of his Table 4 (similar to Attachment IV-A), was recommended unanimously by the RAC as a list for exemption iv. The principles used in creating Dr. Falkow's Table 3 (which is the same principle used to construct Attachment IV-B), was recommended by the RAC (by a vote of 9 to 3) as basis for the exemption iv list. A motion to accept Dr. Falkow's Table 1 with specified modifications (which is very similar to Attachment IV-C), as the exemption iv list, passed by a vote 7 to 5.

There was an opinion expressed by a member of the RAC that pathogens such as Neisseria gonorrhoeae should not be on the list. However, Dr. Adelberg reminded the RAC the concept had been introduced in the proposed revised Guidelines of excluding from the Guidelines non-novel recombinant DNA whether or not CDC Class 2 pathogens were involved. A motion that criteria of genetic exchange be applied in developing the list for exemption iv and that pathogenicity (i.e., CDC Class 1 vs. Class 2) not be considered relevant in this context (recognizing that use of Class 3, 4, and 5 CDC agents is prohibited) passed unanimously.

On the second day, Drs. Helinski and Spizizen presented revised lists based on the same concepts as the three Falkow lists, but embodying some changes in the lists themselves, and the RAC made further slight amendments. The three final alternate lists are attached as Attachments IV-A, IV-B, and IV-C. With each of the alternate lists, any organisms appearing on the list are to be considered "exchangers" and therefore any recombinant DNA molecules composed entirely of DNA segments coming from any members of the list, to be propagated in any member of the list, would be exempted under exemption iv. (One exception to this is in Table IV-C where H. influenzae and H. parainfluenzae form a two-membered list separate from the main 19-membered list.)

Given at the top of each list is a description of the criteria used to construct the list. Thus the Attachment IV-A list is composed of "organisms of the Enterobacteriaceae family which exhibit chromosomal DNA relatedness (20% or more homology of DNA of various pairs tested) and genetic recombination of R-prime (R plasmid carrying chromosomal genes) transfer to E. coli K-12 mediated by the IncP-1 plasmids." The larger Attachment IV-B list includes all the members

of the Attachment IV-A list but adds additional members. All the members of the list "exhibit R-prime (R plasmid carrying chromosomal genes) transfer to E. coli K-12 mediated by the IncP-1 plasmids." The larger Attachment IV-C list contains all the members of the Attachment IV-B list but adds additional members. All members of the list "possess R plasmids (including R plasmids of the IncP-1 group) transferable to E. coli K-12."

Dr. Falkow's handout included tables of data and a list of references. Drs. Helinski and Spizizen promised to send to ORDA a list of published references supporting all the entries on the list in Attachments IV-A, IV-B, and IV-C.

Class v:

The RAC adopted exemption (v).

In considering items to be put under exemption v on the first day of the meeting, it was suggested that cloning of Saccharomyces cerevisiae DNA in E. coli K-12 be a specific case. This was not adopted.

On the second day of the meeting, a motion passed unanimously that "self-cloning" of Saccharomyces cerevisiae be a specific case of exemption from the Guidelines under exemption v.

A composite text indicating all these changes is attached to the Minutes as Attachment V.

- D. Dr. Talbot explained that the suggestion was being withdrawn and the RAC concurred (i.e., there will be no footnote to Prohibition (i) stating that the prohibition of etiologic agents relates only to research in the U.S.).
- E. There was general agreement by the RAC with this concept that institutions receiving NIH funding for recombinant DNA research shall comply with the NIH Guidelines for all their recombinant DNA research independent of the source of funding. It was discussed whether this should be extended even further to all institutions that receive any NIH (or perhaps any HEW) funds, i.e., not only those that receive NIH funds for recombinant DNA research. A motion was accepted in principle that all institutions receiving NIH funds for recombinant DNA research shall perform all their recombinant DNA research, independent of the source of funding, in accordance with the standards of the NIH Guidelines. This motion distinguishes between following the standards of the Guidelines which would be required, and following all the administrative procedures of the Guidelines (MUAs reviewed by ORDA, etc.) which might not be required.

- F. The RAC endorsed retention in the Guidelines of the specification in the description of a P3 laboratory of an autoclave "within the building, and preferably within the controlled laboratory area."
- G. This issue proposes alternative wording of the second sentence of the definition of HVL. The definition of HVL was discussed a number of times on both days of the RAC meeting; discussion of specific cases requesting interpretation of the 1976 Guidelines in regard to using hosts other than E. coli K-12 led the RAC also to consider how these specific cases would be treated in the proposed revised Guidelines, i.e., would they meet the definition of HVL?

The RAC, rather than rewording the second sentence of the definition of HVL as proposed in Question G of Attachment II, preferred instead to delete this sentence. The definition would thus read: "a. HVL. A host-vector system which provides a moderate level of containment."

Other rewording (to change some of the language just preceding the definition of HVL, where biological containment in general is discussed) was proposed but not adopted by the RAC.

Throughout the discussion it became apparent that some host-vector systems might not meet the criteria for HVL but still might be considered by the RAC as safe systems provided they are used under specified containment levels. The proposed revised Guidelines do not discuss this possibility. Therefore, there was strong endorsement for inserting into the Guidelines language which would permit this and other flexibility, i.e., a general flexibility clause.

- H. The RAC, by a vote of 11 to 1, rejected the suggestion in Attachment II of deleting the requirement for HV3 that "the relevant genotypic and phenotypic traits of such HV3 systems have been independently confirmed," i.e., the RAC wishes to retain this requirement.
- I. The RAC expressed willingness to review requests from the private sector (e.g., for interpretations and exceptions from the Guidelines and certification of new host-vector systems). Some members did not really wish to be involved with proprietary data but agreed reluctantly. Dr. Stetten felt that NIH should undertake to review EK2 host-vector systems only if they would be made freely available to other investigators; if a system is truly safer, then not sharing it with others is wrong. Other committee members agreed.

A motion passed unanimously that the RAC expressed its willingness to review proposals from private industry and provide the necessary confidentiality, provided that the submitter agreed to abide by the standards of the NIH Guidelines.

- J. This item was covered during the discussion of viruses (see below).
- K. The RAC accepted the recommendation that the Guidelines provide that the Director, NIH, on the recommendation of the RAC, may designate certain of the agents listed as Class 2 (in the CDC's Classification of Etiologic Agents on the Basis of Hazard) to be considered as Class 1 agents for the purposes of these Guidelines.
- L. The RAC accepted these recommendations and made added suggestions.
- & The Section on "Prokaryotic DNA Recombinants" (i.e., Section
- M. III-B-I-a (2) of the proposed revised Guidelines) should read:

"(2) Prokaryotic DNA Recombinants

- (a) Prokaryotes that exchange genetic information with E. coli. It is expected that prokaryotes that exchange genetic information with E. coli will be exempted from these Guidelines by appearing on the 'list of exchangers' (see exemption iv).

For those not on the list, the containment levels are P1 physical containment + an EK1 host-vector. In fact, experiments in this category can be performed with E. coli K-12 vectors exhibiting a lesser containment (e.g., conjugative plasmids) than EK1 vectors. However, for prokaryotes that are classified as Class 2 (footnote) in reference 5, the containment levels are P2 + EK1.

- (b) Prokaryotes that do not exchange genetic information with E. coli. P2 physical containment + an EK1 host-vector or P1 + EK2 except for DNA from CDC Class 2 (reference 5) agents (footnote) which require P3 + EK2."

- N. The RAC accepted the addition to the Guidelines of a Section dealing with synthetic DNA. The proposed language in Attachment II was accepted with some modifications. This Section would read:

"Synthetic DNAs

"If the synthetic DNA segment can or might yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a

pharmacologically active agent), the containment conditions should be the same as would be used for propagating the natural DNA counterpart.

"If the synthetic DNA sequence codes for a harmless product, it may be propagated at the same containment level as its purified natural DNA counterpart. For example, a synthetic DNA segment, to be propagated in *E. coli* K-12, which corresponds to a non-harmful gene of birds, would require P2 physical containment plus an EK1 host-vector, or P1 + EK2.

"If the synthetic DNA segment is not expressed *in vivo* as a polynucleotide or polypeptide product, the organisms containing the recombinant DNA molecules are exempt from the Guidelines."

- O. The RAC accepted the expansion of the section on "Fungal or Similar Lower Eukaryotic Host-Vector Systems." The proposed language in Attachment II was accepted with one modification. This section, including minor suggestions for clarity added by Dr. Gottesman after the RAC meeting, would then read:

"Fungal or Similar Lower Eukaryotic Host-Vector Systems

"The containment criteria for DNA recombinant experiments using these host-vectors most closely resemble those for prokaryotes, rather than those for the preceding eukaryotes, since the host cells usually exhibit a capacity for dissemination outside the laboratory that is similar to that for bacteria. Therefore, the procedures established for certification of HV systems other than *E. coli* K-12 (Section II-D-2) will also apply to these fungal or similar lower eukaryotic host-vector systems.

"Once approved by NIH, HV1 systems may be used under P2 containment for shotgun experiments with phages, plasmids, and DNA from prokaryotes other than CDC Class 2 agents (footnote), and lower eukaryotes that do not produce polypeptide toxins. Other classes of recombinant DNA experiments with these HV1 systems will require prior approval and classification by NIH. Should HV2 or HV3 systems of this type be developed and approved by NIH, guidelines for their use in other types of recombinant DNA experiments will also be established."

- P. These concepts were accepted by the RAC, i.e., IBC review and & compliance with the standards of the Guidelines would be required
- Q. for all recombinant DNA research at institutions that received any NIH funds for recombinant DNA research (except, of course, for research covered by one of the exemptions from the Guidelines).

- R. This item (research can begin upon IBC approval without ORDA prior approval) was discussed extensively and accepted by the RAC since it would mean less unwarranted delay. Among the questions that were raised was a concern about the number of MUAs approved by IBCs that were later disapproved by ORDA. Dr. Kamely noted that some of these were situations not explicitly covered by the Guidelines and thus required interpretation. Other issues included the possibility of research being initiated that was not in compliance, the inability of NIH to stop funding until the laboratory was actually shown to be in non-compliance, and the fact that not all IBCs would have the same attitude toward self-regulation. One RAC member did note that approval of human subject experimentation was handled effectively at the local level and would set a good precedent.
- S. These items (i.e., ORDA post-review of ongoing projects & the responsibility of the IBC to modify a research protocol when ORDA finds it is in noncompliance with the Guidelines) were endorsed by the RAC. ORDA prereview would still be expected on new grant applications.
- T. These items (i.e., change of name to institutional bio- & safety committee and their mandate to make an independent evaluation of the containment levels required by the Guidelines) were endorsed by the RAC.
- U. This item (i.e., that public membership on the IBC be mandated) was discussed extensively. Dr. Redford suggested that there be at least two public members. Dr. Kutter suggested at least one laboratory technician. Other RAC members objected strongly to NIH telling institutions who should be on their committees. Dr. Adelberg felt it was not an issue on which this scientific committee should be giving advice. A motion by Dr. Redford that it be suggested (not mandated) that institutions give consideration to appointing one or more public members and one or more laboratory technicians to their IBC passed with 7 in favor and 5 opposed.
- V. The RAC rejected the suggestion in Attachment II that "some person or persons be collectively responsible for biosafety monitoring of recombinant DNA research at the P1 through P3 levels and that a special biological safety officer be designated with certain responsibilities at the P4 level." They preferred the language in the proposed revised Guidelines that "each institution in which recombinant DNA research at a P3 or P4 containment level is being conducted shall designate a biological safety officer."

- Y. The RAC accepted this suggestion that the biological safety officer be a member of the IBC.
- Z. The RAC accepted this suggestion (that a section on penalties be included and that it be stated that violation of the NIH Guidelines may result in suspension, limitation, or termination of NIH grants or contracts). Dr. Redford preferred that it state that the suspension, limitation, or termination would be for NIH recombinant DNA research grants or contracts, i.e., not to include totally extraneous grants or contracts.
- AA. The RAC accepted this suggestion of including a registration section.
- BB. The RAC accepted this suggestion to permit voluntary "registration and certification by institutions in the private sector." The concept was reiterated that if NIH is performing a service for industry, we should expect in return their agreement to follow the standards of the Guidelines.
- CC. The RAC accepted the inclusion in the Guidelines of "a provision on disclosure of information that would set general policy guidelines in regard to patents."

### III. EK2 HOST-VECTOR SYSTEMS

#### A. Plasmid Systems

Dr. Adelberg summarized the deliberations of the plasmid Working Group which had met on April 26.

##### 1. $\chi$ 2282

The RAC voted unanimously to recommend approval of  $\chi$ 2282 for use as an EK2 host in place of  $\chi$ 1776 for the cloning of the mouse dihydrofolate reductase gene, provided that an approved EK2 vector is used.

The Committee noted that Dr. Cohen has submitted data showing that the  $\text{thyA}^+$  character has not decreased the sensitivity of  $\chi$ 2282 to DAP deprivation or to bile salts. The  $\text{thyA}^+$  character should not alter the ability of  $\chi$ 2282 to meet criteria for transmissibility, since the transmission tests that are required are done in the presence of thymidine.

2.  $\chi$ 1776 (pSC101) and  $\chi$ 1776 (pCR1)

At its June 1977 meeting, the RAC adopted explicit criteria for host-plasmid systems proposed for EK2 certification. At its October-November 1977 meeting, the RAC passed a motion that the systems  $\chi$ 1776 (pSC101) and  $\chi$ 1776 (pCR1), which were certified prior to these criteria, should be rereviewed by the plasmid Working Group. Additional data on these systems, along with  $\chi$ 1776 (pMB9) as a control, were submitted by Dr. Roy Curtiss. The Working Group concluded that the data show that  $\chi$ 1776 (pCR1) is definitely inferior with regard to transmissibility, and recommended that this system should not be allowed for any new cloning since superior plasmids are available. However, the Working Group recommended that existing clones need not be destroyed because  $\chi$ 1776 (pCR1) is safer than required by the Guidelines for an EK2 system. The RAC voted unanimously to accept this recommendation.

The decision concerning pSC101 (and also pMB9) will be deferred until the Committee can obtain the following data:

<u>Mobilizing Vector</u>	<u>EK2 System</u>
R549 <u>drd1</u>	pMB9
R1 <u>drd19</u>	pSC101
R64 <u>drd11</u>	pBR322

The Working Group is requesting that additional data be obtained because new data from Roy Curtiss show one plasmid which mobilizes pMB9 at a higher rate than previously seen, and another plasmid which does the same for pSC101. The Working Group wants to see these experiments repeated, with controls, before it reconsiders its policy on acceptable limits (which is based on the best performance available), and/or on the status of pMB9 and pSC101. Dr. Nutter was asked to assign this testing to Dr. Clowes. The RAC accepted these recommendations.

3.  $\chi$ 1776 (pOP203-3)

The RAC voted unanimously to accept the recommendations of the plasmid Working Group to request  $r_1 \times r_2$  data from Dr. Giorno before acting on his request that pOP203-3 with  $\chi$ 1776 be certified as an EK2 host-vector system. F-lac should be used as one of the mobilizing plasmids.

The Working Group noted that the insertion of a 203-base pair segment of the lac gene may confer homology with a mobilizing plasmid (and must do so in the case of F-lac),

and therefore may affect transmissibility.  $R_3$  data are not sufficient, according to the "Instructions to Investigators".

4. Modification of Existing EK2 Plasmids to Permit EK3 Testing

The plasmid Working Group has learned that efforts to accomplish modification of existing EK2 plasmids to permit EK3 testing are underway in the laboratories of Drs. Helinski, Curtiss, and Vapnek. It is unlikely that any will be ready until late next fall.

5. The Ou-Anderson manuscript: Dr. Adelberg noted that the claim by Ou and Anderson to have demonstrated  $F^- \times F^-$  recombination (including  $\chi 1776 \times F^-$ ) has been challenged by Dr. Norton Zinder (personal communication), who has obtained evidence that one of their "F" strains carries an unexpressed conjugal plasmid. The Working Group will defer action on testing criteria for  $\chi 1776 \times F^-$  recombination until it has had confirmation of the phenomenon from Drs. Falkow, Curtiss and Low, who are independently experimenting with other  $F^-$  strains. They will look for transfer of a non-conjugal plasmid as well as chromosomal marker recombination, as a more sensitive test.

B. Phage Systems

The report on phage systems was presented by Drs. Campbell and Gottesman.

1. In vitro Packaging

The RAC at its October-November 1977 meeting had approved an in vitro packaging system for use with certified EK2 lambda systems. The Committee had specified criteria, including UV irradiation, that must be satisfied for each packaging extract. Dr. Gottesman discussed the requirements for in vitro packaging systems for lambda phage recombinants in the light of comments by several investigators that UV irradiation should not be required. The RAC felt that UV irradiation need not be a specific requirement, and adopted revised criteria (Attachment VI). A statement on verification of safety features in EK2 vectors bearing cloned segments was also adopted. These requirements will be published in the Bulletin.

2. Modified  $\lambda$ gtWES. $\lambda$ B

The Committee reviewed a request from Drs. Bell and Rutter to use a modification of  $\lambda$ gtWES. $\lambda$ B as a certified EK2 system. The EK2 vector has been slightly modified so that segments can be joined by dA-dT tailing and thus obviate the need to

use Eco RI. The Committee considered this to be a minor modification and recommended approval of the general principle that is joining a cloned segment to the vector of an EK2 system, dA-dT tailing can be substituted for restriction enzyme joining without special approval.

### 3. Charon 21A

The Committee reviewed an application from Dr. Frederick Blattner's laboratory for EK2 certification of a host-vector system based on phage Charon 21A. Charon 21A is a recombinant between  $\lambda$ gtWES- $\lambda$ B and Charon phages, and can be considered to be an improved modification of Charon 16A which is certified for use in EK2 systems. It appears to satisfy all the requirements for EK2 phage systems, but had not been considered in detail by all members of the subcommittee because of the late date of submission of the application. The RAC voted to recommend approval of Charon 21A subject to confirmation by mail ballot from the subcommittee.

### 4. Production and Testing Contracts for EK2 Systems

Dr. John Nutter of NIAID described the production and testing contracts awarded for EK2 and EK3 systems. In 1976, NIAID let four contracts for the development of EK2 systems. The contractors were: Fred Blattner for the production of the Charon phages 3A, 4A, 16A; Donald Helinski for plasmid vectors; Dan Ray and Fred Wilcox for the development of a three component M13 vector (this contract has recently received a no cost extension) and Roy Curtiss for the development of  $\lambda$ 1776 and other E. coli hosts. All these contracts have or will terminate in the near future.

At the same time contracts were awarded for the testing of EK2 systems and their possible elevation to EK3. The contractors were independently to verify the genotype and phenotype of the host and vector and then to determine their survivability. Verification was to be done by Dr. Dodge for E. coli hosts  $\lambda$ 1776 and DP50, Dr. Lieb for lambda phages, and Dr. Clowes for plasmid vectors. Drs. Preter and Levy were to determine baseline data under culture conditions, and in mice and humans. A contract has also been awarded to test simulated accidental spills. A sewage testing contract is still to be negotiated.

A third type of contract was awarded to Josephine Curtiss at the University of Alabama to propagate and package EK2 systems. The material will be returned to NIH for distribution. A announcement of availability will appear in the next issue of the Bulletin.

The RAC went on record that  $\lambda$ 1776 (pBR322) should be tested for EK3 properties.

IV. CONSIDERATION OF MINUTES OF PREVIOUS MEETING

Dr. Helinski requested that Page 4 of the Minutes of the October-November, 1978 meeting be modified to indicate that the Falmouth meeting had served as a forum for the exchange of previously published data on the ecology of E. coli. The Minutes were approved subject to this and several other minor modifications.

V. REQUESTS FOR REDUCTION OF CONTAINMENT LEVELS FOR CHARACTERIZED CLONES

- A. The Committee reviewed a request by Dr. Donald Brown for approval to lower the containment levels to P2 + EK1 for characterized clones containing the 5SDNA from Xenopus, and to introduce minor modifications in the clones by reaction with sodium bisulfite or S1 nuclease. The Committee noted that deletions or substitutions produced by chemical reaction would not drastically alter the DNA. The Committee approved the reduction of containment levels for the characterized clones to P2 + EK1, and unanimously passed a motion that the clones can continue to be handled under P2 + EK1 conditions after chemical treatment as described in the request.

Dr. Rowe then made a motion that investigators who wish to modify characterized clones by mutagenesis or by minor substitutions or insertions need not obtain approval from ORDA before working at lowered containment levels. In such cases, approval by the IBC is recommended but not required. The motion was passed unanimously.

- B. A request by Dr. Ronald Reeder to lower the containment level for a series of clones from Xenopus was considered. The request includes a series of rigorously characterized clones of ribosomal genes and subclones thereof, the cloning of ribosomal DNA of greater than 99% purity from the somatic cells of a single frog, the cloning of histone genes from Xenopus embryos, and the cloning of genes from Xenopus oocytes. The RAC unanimously approved all of the requests at the P2 + EK1 levels of containment.
- C. A request from Dr. Robert Roeder to allow the propagation of a hybrid vector composed of lambda phage and a 3000 base segment from Xenopus containing tRNA genes was approved by the RAC at P2 + EK1 containment. Most of the Xenopus insert consists of spacer which was not considered by the Committee to be harmful.
- D. A request by Dr. Sherman Weissman to lower the containment level to P2 + EK1 for characterized plasmids JW101 and JW151 containing human and gamma globin DNA was unanimously approved. The inserts are 800 bases long and partial sequencing data on 50 bases shows

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compatibility with the amino acid sequence. Analysis with restriction endonucleases shows the expected pattern of fragments.

- E. A request by Dr. Carl Schildkraut to propagate characterized plasmids (ZMoG and ZM $\beta$ G) containing cDNA copies of mouse  $\alpha$  and  $\beta$ globin DNA was unanimously approved at P2 + EK1. The partial nucleotide sequences correspond to the amino acid sequence, the restriction pattern is consistent, and the plasmid DNA hybridizes to mouse globin DNA.
- F. A similar request by Dr. Ralph Giorno to propagate characterized plasmids containing mouse  $\alpha$  and  $\beta$  globin DNA under P2 + EK1 conditions was also unanimously approved contingent upon either the demonstration that E. coli strain N543 does not have conjugative plasmids or that a host which meets EK1 criteria is used.
- G. Dr. John Baxter and colleagues requested permission to reduce containment levels from P3 + EK2 to P3 + EK1 or P2 + EK2 for a series of clones containing all or part of the gene for rat growth hormone. Nucleotide sequence and restriction analysis data indicates correspondence with the amino acid sequence. The question of potential harm if the gene were to be expressed was raised by Dr. Helinski. Dr. Gottesman indicated that the amount of growth hormone produced in E. coli would not affect human hormonal balance. Other evidence indicates that rat hormone does not cross-react with human and that growth hormone is totally inactive when administered orally. The RAC unanimously voted approval for P3 + EK1 or P2 + EK2 containment levels.

Later Dr. Gottesman made a motion to permit lowering of containment levels for characterized rat growth hormone clones to P2 + EK1. The motion passed 8 to 2.

- H. A second request from Dr. Baxter to propagate characterized clones of human growth hormone and human somatomammotropin (related to growth hormone) at P2 + EK1 containment levels was approved on the grounds that the inserts were characterized. The nucleotide sequence for amino acids 24-190 has been determined. The RAC unanimously voted approval of P3 + EK1 or P2 + EK2 containment levels for these clones.

A second motion to lower the containment requirements to P2 + EK1 for characterized clones of human growth hormone resulted in a vote of six for and six against. Dr. Stetten voted no and the motion was defeated.

- I. A request by Dr. Howard Goodman to propagate characterized clones of rat insulin DNA under P2 + EK1 conditions was approved by a vote of

12 to 1. The RAC specified that the approval for reduction in containment was restricted to the clones specifically cited in the MUA.

- J. A request by Dr. Robert Goldberger to propagate a plasmid containing cDNA made from vitellogenin mRNA was approved at P3 + EK1 (Dr. Gottesman abstaining) provided that the vector pBR322 was used. The mRNA had been characterized by hybridization, gel electrophoresis, and translation in a cell-free system.
- K. A request by Dr. Robert J. Crouch to lower containment for a well-characterized clone containing mouse ribosomal DNA to P2 + EK1 was approved. The DNA had been characterized by hybridization and restriction enzyme analysis.

#### VI. REMARKS OF THE CHAIRMAN

Dr. Stetten addressed the Committee regarding his decision to resign as its chairman. His remarks are reproduced in Attachment VII.

#### VII. VIRUS WORKING GROUP REPORT

The Virus Report was presented by Dr. Harry Ginsberg. Dr. Ginsberg described the events leading to the U.S. - EMBO Workshop to Assess Risks for Recombinant DNA Experiments Involving the Genomes of Animal, Plant, and Insect Viruses. He noted that discussion at the meeting of the NIH Director's Advisory Committee in December 1977 centered about the restrictions imposed by the revised Guidelines upon recombinant DNA research when viruses are used. As a result of the discussion, NIH sponsored the virus workshop at Ascot, England, in January 1978. The participants came from a variety of disciplines and included molecular biologists, virologists, epidemiologists and experts in infectious disease. Most were not involved in recombinant DNA research. The charge to this group was to assess the possibility of hazard arising from the cloning of viral DNA and from the use of viral DNA as a vector. They were to consider the hazard to laboratory workers and to the population at large.

The workshop considered the entire range of eukaryotic viruses, including insect and plant pathogenic viruses. No situation was envisioned in which a recombinant virus or variant would become a greater hazard than the virus itself. In a number of instances, the group believed that the host range would be altered but that there would be no increase in pathogenic potential. The report represents the unanimous opinion of the participants at the Workshop.

Subsequent to the U.S. - EMBO Workshop, a Working Group was convened to review the findings and translate them into recommendations for changes in the experimental section of the Guidelines. Their

analysis stems from the present knowledge of the structure of viral genomes and their mode of replication.

- A. The Working Group recommended that mouth pipetting be prohibited at the P1 physical containment level. This evoked considerable discussion by the RAC. It was the consensus of the RAC that many experiments classified as P1 need not include a ban on mouth pipetting, and that therefore P1 in general should not be redefined. However, the RAC by a vote of 10 to 2 approved the principle that P1 with an added prohibition on mouth pipetting should be endorsed for designated experiments.
- B. The Working Group recommended for certain classes of experiments the use of *E. coli* K-12 with "non-mobilizable plasmid vectors," i.e., a level of containment falling between EK1 and EK2. Dr. Adelberg pointed out that the terminology was incorrect and rather than speaking of "non-mobilizable vectors" one should speak of "vectors certified for use in EK2 systems." With this change the RAC unanimously endorsed this new level of biological containment to be used in specific instances.
- C. It was pointed out that acceptance of the Working Group recommendations would remove from the current prohibited experiments both the use of vesicular stomatitis virus (a Class 3 CDC virus), and all of the oncogenic viruses classified by NCI as moderate risk agents. There was no objection to this by the RAC. It was pointed out that the NCI list is outdated and was conservative.
- D. The RAC then discussed Table 1 and Table 2 of the Working Group Report and the accompanying text describing the table. This text would be inserted into the Guidelines. The inclusion of the tables in the Guidelines was also recommended but not mandatory. The RAC made some changes in the texts and tables, and then unanimously adopted them. (Attachments VIII-A and VIII-B).

The effect of this is as follows:

Section III-B-1-a-(1)-(g), "Cloning of Viral Genomes from Eukaryotic Cell DNA" of the proposed revised Guidelines, is eliminated. Section III-B-1-b-(1), "Viruses of Eukaryotes" is eliminated; substituted for it is the text attached to the Minutes as Attachment VIII-A. In section III-B-3, "Experiments with Eukaryotic Host-Vectors," subparts a-"Vertebrate Host-Vector Systems," and b-"Invertebrate Host-Vector Systems," are eliminated; substituted is text attached to the Minutes as Attachment VIII-B, which consists of three subparts: a-"Vertebrate Host-Vector Systems"; b-"Invertebrate Host-Vector Systems in Which Insect Viruses Are Used to Propagate Other DNA Segments"; and c-"Plant Viral Host-Vector Systems."

VIII. PROTOCOLS FOR REQUIRED CONTAINMENT

Below are described the actions taken by the RAC on proposed protocols for required containment levels and for compliance with the 1976 Guidelines.

A. Cloning of Bovine Satellite DNA

Dr. Susan Gerbi of Brown University requested NIH review of a proposal to clone 99% pure bovine satellite DNA under P2-EK2 conditions. The Guidelines permit a one-step decrease in physical or biological containment in relation to the conditions required for the corresponding shotgun experiment when the DNA to be inserted has been enriched to 99% purity by physical and chemical techniques, and is free of harmful genes. Shotgun conditions for cloning bovine DNA are P3-EK2. Therefore, "purified" bovine DNA can be cloned under P2-EK2 conditions if the DNA is 99% pure and free of harmful genes.

The RAC agreed that the evidence for 99% purity is acceptable. There was some discussion about whether the DNA preparation could be said to be free of harmful genes in view of the fact that the functions of satellite DNA are unknown. However, it was pointed out that, in all cases investigated, satellite DNA is not translated. Therefore, it is reasonable to take the position that such DNA is free of harmful genes. The RAC then voted unanimously to approve the requested containment levels of P2-EK2.

B. Cloning in Pseudomonas putida

The RAC reviewed requests from Dr. James Shapiro of the University of Chicago, and Dr. J. G. Vacca, on behalf of Dr. Chakrabarty, of the General Electric Company to use Pseudomonas putida as a host for recombinant DNA experiments.

In one set of experiments, Dr. Shapiro wishes to clone P. putida DNA using E. coli as a host. In a second set of experiments, Dr. Shapiro requests permission to use P. putida as a host for cloning. There are two subsets to the latter experiments. In one, Dr. Shapiro wishes to conduct self-cloning experiments. The P. putida DNA carried by plasmids R1162 or RP4 1 and previously cloned in E. coli would be returned to P. putida for propagation. In the second subset of experiments involving P. putida as a host, Dr. Shapiro intends to develop cloning vectors with a wide host range that could be propagated in either E. coli or P. putida. The hybrid vectors would be from Col E1 and R1162 or RP4 1. The vectors could be grown in E. coli under P1 conditions since both parental vectors grow in E. coli.

Dr. Chakrabarty is interested in conducting two types of experiments. In the first, he wants to clone P. putida genes in P. putida using P. putida plasmids as vectors. There would be two kinds of vectors, PR4 1, a nonconjugative plasmid, and RPl, a conjugative plasmid. In the second experiment, Dr. Chakrabarty wants to substitute E. coli or pMB9 DNA for P. putida DNA as a donor. Dr. Chakrabarty suggests P1 containment when the nonconjugative plasmid is used and P2 when the conjugative plasmid is employed.

The RAC agreed that, according to the 1976 Guidelines, shotgun cloning of P. putida DNA into E. coli K-12 can be conducted under P1-EK1 conditions or even with conjugative plasmids under P1 conditions.

The RAC felt that using P. putida as a host for E. coli DNA is not permitted under the 1976 Guidelines because of the requirements that the host must be comparable to EK1 in biological containment, i.e., free from conjugative plasmids. However, the RAC voted 9 to 1 to recommend that an exemption be granted to allow all of the requested experiments because P. putida and E. coli exchange DNA, and such pairs of organisms have been recommended for total exemption under the proposed revised Guidelines.

The RAC was then specifically asked to judge which of the proposed experiments could be approved under the 1976 Guidelines. The Committee noted that to be comparable to EK1 the P. putida strains would have to be enfeebled and free of conjugative plasmids.

The RAC then unanimously passed a motion to the effect that the investigators should be informed that, under the 1976 Guidelines, approval can be given under P1 conditions for those experiments in which no conjugative plasmids are present in the P. putida host when recombinant DNA is present, and after data have been submitted on (the poorer) survival in soil of the P. putida host strain compared to wild type. If these two conditions cannot be met, the RAC recommends that an exemption to the 1976 Guidelines be granted on the grounds that P. putida and E. coli exchange DNA, and that these experiments have been recommended for total exemption under the revised Guidelines.

#### C. In vitro Manipulation of Recombinant DNA Molecules

The RAC reviewed a statement on the in vitro handling of recombinant DNA prepared by the Harvard University Committee on the Regulation of Hazardous Biological Agents. The statement establishes different requirements for the in vitro handling of recombinant DNA depending on whether the in vivo requirements are P1-EK1, or whether the in vivo conditions require P2 or P3 containment when in an EK1 or EK2 host.

The RAC did not vote on the specific proposals of the Harvard Committee although some members thought the proposed requirements to be overly stringent. Rather, the RAC unanimously passed a motion that the handling of naked DNA normally requires P1 conditions. Furthermore, cleavage of the recombinant DNA with restriction endonucleases, as in the proposed addendum, unequivocally removes such experiments from any restraints of the Guidelines.

D. Cloning of Chemically Synthesized Genes

The RAC reviewed proposals by Dr. Ray Wu of Cornell University for the setting of containment levels for the cloning of chemically synthesized genes for human insulin chains. Dr. Wu wrote proposing to clone a chemically synthesized insulin A gene under P1-EK1 conditions, and a synthetic insulin B gene under P1-EK2 or P2-EK1 conditions.

The RAC concluded that the containment levels for these experiments should be analogous to those required for a characterized clone derived from a shotgun experiment. In the case of a human DNA sequence, P2-EK1 conditions can be approved when the cloned recombinant has been rigorously characterized and is free of harmful genes. The RAC voted 9 to 1 to approve the request to clone synthetic genes for human insulin A and B chains under P2-EK1 or P1-EK2 conditions. The negative vote was cast because of concern about possible hormonal or antigenic activities of the insulin A and B chains.

E. Cloning Eukaryotic DNA in Yeast

The RAC reviewed requests from Dr. Ronald Davis of Stanford University and Dr. Richard Firtel of the University of California, San Diego, to clone eukaryotic DNA in Saccharomyces cerevisiae.

Dr. Davis requests approval to clone in yeast Drosophila melanogaster DNA sequences linked to an E. coli non-conjugative plasmid or DNA linked to a selectable yeast gene. The recombinant first will be constructed in E. coli and then transferred to yeast. Dr. Davis requests approval for use of P2 conditions for these experiments.

Dr. Firtel requests approval for the cloning in yeast of Dictyostelium discoideum DNA sequences linked to regions of the yeast genome containing selectable markers linked to non-conjugative E. coli plasmids. The triple recombinants first will be formed in E. coli and then used to transform yeast strains deficient in the selectable markers. Approval of P2 conditions is requested.

Members of the RAC felt that there was little doubt about the

safety of the newly proposed experiments. However, concern was expressed that the yeast hosts, in general, cannot be considered to be contained as they are capable of surviving in nature. The opinion was expressed that the proposed experiments appear to be exceptions to the 1976 Guidelines, and that perhaps the experiments should be deferred until yeast strains with reduced survival in nature are identified. A proposal was made that perhaps the concept of an experiment "being safe" should be an alternative to the requirement for biological containment in the host.

The RAC then voted unanimously to approve the requests of Drs. Davis and Firtel subject to the submission of appropriate MUAs. It is the sense of the RAC that ORDA should use these decisions as precedents for approving the insertion of DNA sequences from other lower eukaryotes into S. cerevisiae.

F. Transfer of DNA to Phycomyces

ORDA asked the RAC to review an MUA submitted by Dr. Max Delbruck of the California Institute of Technology. The MUA proposes three series of experiments under P1 conditions:

1. Transfer of yeast 2  $\mu$  DNA circles to Phycomyces.
2. Transfer into Phycomyces of yeast 2  $\mu$  DNA circles with insertion of an E. coli gene coding for a suitable enzyme marker.
3. Transfer into Phycomyces of yeast 2  $\mu$  DNA circles with insertions of Phycomyces genes.

The RAC discussed these experiments at the same time as the experiments described in item E (above) were considered, and many of the comments made by the RAC apply also to these experiments. The RAC unanimously voted to approve the experiments in Phycomyces based on the precedent of approval of self-cloning experiments in yeast.

G. Propagation of Recombinant Plasmids in Plant Pathogens

Dr. Nicholas Panopoulos of the University of California, Berkeley, asked the RAC to review an MUA dealing with an investigation of the ability of various recombinant plasmids to replicate in plant pathogenic bacteria. The immediate objective of the research is to test various plasmid vectors for the ability to replicate in certain species of phytopathogenic bacteria, the ability to be mobilized from E. coli hosts into these species, and the ability to transform these species. The bacterial hosts to be used include Pseudomonas species, Erwinia species, and Xanthomonas species. The long-term objective is to develop host-vector systems for certain species of plant pathogenic bacteria. The

plasmids to be used in the initial studies were constructed by in vitro techniques and, therefore, constitute recombinant plasmids.

Members of the RAC felt that the proposed work will not generate any hazard, and that most, if not all, of these experiments will be excluded from the revised Guidelines under exemption (iv) for genetic exchangers. The RAC then unanimously passed a motion to ask the Director, NIH, for an exception to the current Guidelines in order to carry out the experiments described (except those previously approved) because the introduction into plant pathogens of E. coli plasmids restructured by recombinant DNA techniques is unlikely to pose any additional hazard. As noted above, the NIH has already approved the introduction of EK2 vectors, constructed by recombinant DNA techniques, into plant pathogens.

#### H. Cloning B. subtilis and E. coli DNA in B. subtilis

The RAC reviewed an MUA submitted by Dr. Frank Young of the University of Rochester dealing with an experiment in which a chimeric plasmid composed of B. subtilis chromosomal or phage DNA linked to E. coli plasmid pMB9 is to be cloned in B. subtilis.

The NIH has already approved at the P1 level experiments in which S. aureus or B. subtilis plasmids are used to clone B. subtilis chromosomal or phage DNA in B. subtilis.

The RAC voted unanimously to approve at the P2 level the following experiments provided that non-reverting asporogenic host strains with low survival are utilized, and that data on these strains are submitted to the NIH:

1. The cloning in B. subtilis hosts of B. subtilis DNA on E. coli plasmids or phages;
2. The cloning in B. subtilis hosts of E. coli DNA on E. coli plasmids or phages.

#### I. Recombinant DNA Research with Bacillus popilliae and Bacillus thuringiensis

Dr. Robert Faust of the Beltsville Agricultural Research Center, USDA, requested NIH review of a research proposal involving recombinant DNA research with Bacillus popilliae and Bacillus thuringiensis.

Dr. Faust proposes to construct hybrid plasmids from B. thuringiensis chromosomal DNA and its indigenous plasmid, and propagate them in B. popilliae. Transformed strains will be identified by antibiotic resistance or added fermentation ability.

Dr. Faust discusses in some detail the relationship of this host-vector system to E. coli and other prokaryotic systems described in the Guidelines. He states that the narrow host range and lack of evidence for pathogenicity in mammals (i.e., Class I agent) should provide biological containment equal to Ekl hosts.

Dr. Faust cites other authors who maintain that B. popilliae and B. thuringiensis exchange genetic information. He is unable to say whether the B. thuringiensis plasmids are non-conjugative.

The RAC noted that these organisms are safe pesticides registered by the EPA. However, it was felt that these organisms will have difficulty in meeting HVI criteria under the proposed revised Guidelines. In addition, these experiments may not be exempt under the revised Guidelines because this pair of organisms may not appear on the list of genetic exchangers. The RAC then voted 10 to 1 to recommend that an exception be granted for the proposed recombinant DNA experiments based on their safety rather than on containment.

#### J. Cloning in Rhodopseudomonas capsulata

Dr. Alan Lambowitz of Saint Louis University requested review of recombinant DNA experiments involving Rhodopseudomonas capsulata.

The experiments are divided into two stages. In the first stage, fragments of Rhodopseudomonas capsulata DNA would be cloned in Rhodopseudomonas capsulata strain MB615 using E. coli plasmids as vectors under P2 conditions. A clone will be selected that carries a fragment of the R. capsulata genome that can complement the lesion that renders MB615 non-photosynthetic.

In the second stage, the plasmid thus selected will be isolated from R. capsulata and cloned in an Ekl host. This plasmid will then be amplified and harvested to produce a probe for use in in vitro RNA-DNA hybridization experiments.

The information which was submitted contained no data on survival outside the laboratory. RAC members felt that the situation parallels the Pseudomonas putida case described in item B. The RAC unanimously passed a motion to the effect that the investigators should be informed that, under the 1976 Guidelines, approval can be given under P1 conditions for those experiments in which no conjugative plasmids are present in the R. capsulata host when recombinant DNA is present, and after data have been submitted on (the poorer) survival in soil of the R. capsulata host strain compared to wild type. If these two conditions cannot be met, the RAC recommends that an exemption to the 1976 Guidelines be granted on the grounds that R. capsulata and E. coli exchange DNA, and that similar experiments have been recommended for total exemption under the revised Guidelines.

K. Cloning a Region of SV40 into E. coli K-12

Dr. Robert Tjian of the Cold Spring Harbor Laboratory submitted a request to clone DNA sequences which lie within a segment of SV40 DNA which does not encode any known gene products but is likely to contain part or all of the origin of SV40 replication. It is proposed to insert these fragments into an EK2 host-vector system under P3 conditions.

The RAC felt that the 1976 Guidelines appear to require P4-EK2 or P3-EK3 conditions, because the segments to be inserted, although purified, were not cloned and, therefore, do not meet the requirement that the segment must be cloned prior to lowering the required containment to P3-EK2 conditions. However, members of the RAC felt that the degree of purification of the segments to be inserted is likely to be equivalent to that achievable by cloning. The RAC voted unanimously that an exception should be granted to conduct these experiments under P3-EK2 conditions if certain additional information on the degree of purification is available.

L. Cloning in Salmonella typhimurium

The RAC reviewed a request from a group of eleven scientists to permit recombinant DNA experiments involving the introduction of S. typhimurium and/or E. coli DNA into S. typhimurium.

The RAC approved the Salmonella experiments on the basis that it is their interpretation of the Guidelines that S. typhimurium strain LT2 can be considered as a host equivalent to E. coli K-12 for the purposes of cloning S. typhimurium or E. coli DNA. The RAC then unanimously passed a motion that experiments which involve the transfer of S. typhimurium DNA cloned in E. coli back into S. typhimurium LT2 should be approved under P2 conditions.

IX. WORKSHOP ON RISK ASSESSMENT OF AGRICULTURAL PATHOGENS

Dr. Day presented the report of the Workshop on Risk Assessment of Agricultural Pathogens held on March 20-21, 1978. A number of the recommendations of the Workshop Report had already been adopted by the RAC earlier in the meeting. Additional changes in the Guidelines recommended in the Workshop Report and approved unanimously by the RAC were the following:

- A. A motion to include in the Guidelines, as part of Appendix B, the "Hazard Classification of Plant Pathogens," as taken from the Workshop Report, was approved with a minor addition proposed by Dr. Day. The material to be added to Appendix B of the proposed revised Guidelines is attached to the Minutes as Attachment IX.

- B. Section III-B-1-a-(1)-(e) on "Other Cold-Blooded Animals and Lower Eukaryotes." Under item 2, the first sentence in the proposed revised Guidelines is: "2. The remainder of the species in this class: P2 + EK1 or P1 + EK2." Change this to: "2. The remainder of the species in this class, including plant pathogenic or symbiotic fungi that do not produce potent toxins: P2 + EK1 or P1 + EK2."
- C. Section III-B-1-a-(1)-(f) on "Plants." The first two sentences in the proposed revised Guidelines are: "(f) Plants. P2 physical containment + an EK1 host-vector or P1 + EK2. If the plant source carries a known pathogenic micro-organism or makes a potent polypeptide toxin (footnote), the containment must be raised to P3 physical containment + an EK2 host-vector."
- The change is to delete from the second sentence the words "carries a known pathogenic micro-organism or."
- D. Section III-B-3-c on "Plant host-vector systems" in the proposed revised Guidelines will now become Section III-B-3-d to be called "Plant host-vector systems other than plant viruses."

#### IX. OTHER CHANGES IN THE PROPOSED REVISED GUIDELINES

At the conclusion of the meeting, the RAC discussed other changes in the proposed revised Guidelines. In its deliberations, the RAC took into consideration the previously voted upon alterations in the Virus Section.

##### A. Shotgun of Primate DNA into E. coli K-12

One of the reasons given originally for the high containment level for shotgun experiments involving primate DNA into E. coli K-12 was the possible inadvertent cloning of viral DNA. In view of the adoption of the recommendations of the Virus Working Group to lower containment for deliberate cloning of viral DNA, the RAC reconsidered primate shotgun levels, and voted unanimously to rewrite Section III-B-1-a-(1)-(a), as follows (although the exact wording was not voted upon):

"(a) Primates. P2 physical containment + an EK2 host-vector. However any lowering of containment below these levels (i.e., for purified DNA or characterized clones) cannot be made solely by an institutional biosafety committee but requires NIH approval." [Note: It was not discussed at the RAC meeting, but additional text and cross references will now be put into Section III-B-1-c "Lowering of containment levels of characterized or purified DNA preparations and clones"-to remind the reader that primate shotguns are to be treated differently.]

B. "Self-Cloning in Prokaryotic Hosts that Exchange Genetic Information with E. coli"

Wording to be inserted into the proposed revised Guidelines to allow "self-cloning" experiments in prokaryotic hosts that exchange genetic information with E. coli, using E. coli K-12 as an "intermediate host," at P1 physical containment was read to the RAC by Dr. Gottesman and approved unanimously. After the RAC meeting, the text was reworded for clarity. The text, to be inserted under Section III-B-2, "Experiments with other prokaryotic host-vectors," would read:

"It is expected that prokaryotes that exchange genetic information with E. coli will be exempted from these Guidelines by appearing on the "list of exchangers" (see exemption iv). For a prokaryote which exchanges genetic information with E. coli but which is not on the list (Host A), the following experiments may be carried out under P1 conditions without Host A having been approved as an HVI host:

DNA from Host A may be cloned into a vector and propagated in E. coli K-12 under P1 conditions. Subsequently, this recombinant DNA may be returned to Host A by mobilization or transformation, and then may be propagated in Host A under P1 conditions."

X. DATES OF FUTURE MEETINGS

The Committee selected August 2-3, 1978, and October 30-31, 1978, as dates of future meetings.

XI. ADJOURNMENT

The meeting was adjourned at 4:00 p.m., April 28, 1978.

Respectfully submitted,

  
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.

9/5/78  
Date

  
DeWitt Stetten, Jr., M.D., Ph.D.  
Chairman, Recombinant DNA Molecule  
Program Advisory Committee  
National Institutes of Health

CHAIRMAN

VICE CHAIRMAN

STETTEN, DeWitt, Jr., M.D., Ph.D. (79)  
Deputy Director for Science  
Office of the Director  
National Institutes of Health  
Bethesda, Maryland 20014  
301 496-1921

JACOBS, Leon, Ph.D. (79)  
Associate Director for  
Collaborative Research  
Office of the Director  
National Institutes of Health  
Bethesda, Maryland 20014  
301 496-3111

-----

ADELBERG, Edward A., Ph.D. (78)  
Professor  
Department of Human Genetics  
School of Medicine  
Yale University  
New Haven, Connecticut 06510  
203 436-0821

LITTLEFIELD, John W., M.D. (78)  
Professor & Chairman  
Department of Pediatrics  
Children's Medical & Surgical Center  
Johns Hopkins Hospital  
Baltimore, Maryland 21204  
301 955-5976

CAMPBELL, Allan M., Ph.D. (80)  
Professor  
Department of Biology  
Stanford University  
Stanford, California 94305  
415 497-1170

REDFORD, Emmette S., Ph.D., LL.D. (79)  
Ashbel Smith Professor of  
Government and Public Affairs  
Lyndon B. Johnson School of  
Public Affairs  
University of Texas at Austin  
Austin, Texas 78712  
512 471-4127

DAY, Peter R., Ph.D. (80)  
Chief  
Division of Genetics  
Connecticut Agricultural  
Experiment Station  
New Haven, Connecticut 06504  
203 789-7258

ROWE, Wallace P., M.D. (79)  
Chief, Laboratory of Viral Diseases  
National Institute of Allergy &  
Infectious Diseases  
National Institutes of Health  
Bethesda, Maryland 20014  
301 496-2613

GOTTESMAN, Susan K., Ph.D. (81)  
Senior Investigator  
Laboratory of Molecular Biology  
National Cancer Institute  
National Institutes of Health  
Bethesda, Maryland 20014  
301 496-3524

SETLOW, Jane K., Ph.D. (78)  
Biologist  
Brookhaven National Laboratory  
Upton, Long Island, New York 11973  
516 345-3420

HELINSKI, Donald R., Ph.D. (78)  
Professor  
Department of Biology  
University of California, San Diego  
La Jolla, California 92037  
714 452-3638

SPIZIZEN, John, Ph.D. (79)  
Member and Chairman  
Department of Microbiology  
Scripps Clinic & Research Foundation  
La Jolla, California 92037  
714 455-9100 x360

HORNICK, Richard B., M.D. (81)  
Professor and Director  
Division of Infectious Diseases  
School of Medicine  
University of Maryland  
Baltimore, Maryland 21201  
301 528-7562

WALTERS, LeRoy, Ph.D. (80)  
Director  
Center for Bioethics  
Kennedy Institute  
Georgetown University  
Washington, D.C. 20057  
202 625-2371

KUPFER, Elizabeth M., Ph.D. (79)  
Member of the Faculty  
in Biophysics  
The Evergreen State College  
Olympia, Washington 98505  
206 866-6719

ZAITLIN, Milton, Ph.D. (80)  
Professor  
Department of Plant Pathology  
Cornell University  
Ithaca, New York 14853  
607 256-3105

EXECUTIVE SECRETARY

GARTLAND, William J, Jr., Ph.D.  
Health Scientist Administrator  
National Institute of General  
Medical Sciences  
National Institutes of Health  
Bethesda, Maryland 20014  
301 496-6051

RECOMBINANT DNA MOLECULE PROGRAM ADVISORY COMMITTEE

LIAISON REPRESENTATIVES

DUDA, George, Ph.D.  
Division of Biomedical and  
Environmental Research  
Department of Energy  
Washington, D.C. 20545  
301 353-3651

HEDRICH, Richard, Ph.D.  
Coordination Program of Science  
Technology & Human Value  
National Endowment for the Humanities  
Washington, D.C. 20506  
202 382-5996

FULKERSON, John F., Ph.D.  
Cooperative State Research Service  
U. S. Department of Agriculture  
Washington, D.C. 20250  
202 447-4175

LEWIS, Herman W., Ph.D.  
Division of Biological and  
Medical Sciences  
National Science Foundation  
Washington, D.C. 20550  
202 632-4200

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

SELECTED ISSUES FOR COMMITTEE REVIEW

I. Introduction: Definition and Exemptions

The definition and exemption for "non-novel" exchangers evoked much public comment in which a number of recommendations were made. For example, it was urged that the definition be expanded to include segments of chemically synthesized DNA. Much of the comment concerning the exclusion for non-novel exchangers pertained to the proposed standards and procedures for developing a list.

The public views concerning the definition and exemption included:

- that safety rather than novelty should be the criterion for exclusion;
- that the criteria for determining novelty were not explicit;
- that the list of non-novel exchangers should not be limited to the exchange of chromosomal DNA but should also include plasmid exchange (others, however, urged that the list not be broadly drawn at the species level but should deal with exchange at the subspecies level);
- that experiments classified as P1 + EK1 should be exempted from the Guidelines;
- that the proposed revised definition would appear not to permit "self-cloning" experiments.

Recommendations on the process for developing a list included:

- that the burden of proof be on the Director to compile a list of

- novel exchangers that are subject to the Guidelines;
- that the procedures and criteria used in the development of the list be explained thoroughly and that adequate opportunity be given for public review and comment;
  - that all data pertaining to the inclusion of each entry on the list be available for public review.

#### ISSUES FOR THE COMMITTEE'S CONSIDERATION

The extensive public comment on this section included recommendations that there be a general applicability, exemption, and definition section. Accordingly, I am suggesting that such a section be included in Part I, the Introduction. Further, I propose that the section on prohibited experiments be transferred from Part III to Part I. Thus, Part I would establish the jurisdiction of the Guidelines, their applicability, and the experiments that are prohibited and exempt.

The following suggestions also arise from my review:

- A. • In your Committee's proposed revision, the purpose of the Guidelines is to "establish procedures for handling organisms and viruses containing recombinant DNA molecules." In the current Guidelines the purpose speaks to "research with recombinant DNA molecules." It was noted in our review that your recommendation might inadvertently have the effect of removing from the list of prohibited experiments those in which recombinant DNA molecules were created from the DNA of certain pathogens even though this DNA was not contained within an organism. Because

there was strong public support for retaining the prohibition, would you object if the section were to specify procedures for constructing and handling (a) recombinant DNA molecules and (b) organisms and viruses containing recombinant DNA molecules?

Thus, in the exemption section, most recombinant DNA molecules outside of organisms and viruses that are not covered under the prohibited experiments would be exempt from the Guidelines.

- B. • In light of the comments on the definition, I am considering one that would not include the phrases "the capacity to infect" and "natural physiologic processes." A substitute definition would speak to "molecules that have been constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate or be integrated into the genome of a living cell." I would appreciate your views on this suggestion, including the addition of "synthetic DNA segments" to the definition.
- C. • In light of the comments on the criteria and procedures for a listing of non-novel exchangers, new language has been drafted for experiments to be exempted from the Guidelines. A copy of a letter with the NIH draft on exemptions that I sent to Dr. Spizizen and his Working Group is enclosed for your review. Please note that no prohibited experiments may be exempt. To emphasize the importance of this concept, prohibited experiments would be transposed from Section III to Section I and would appear before the exempted experiments. I would

appreciate your comments on this draft and the transposition of prohibited experiments to this section. In light of these modifications, your Committee's revisions speaking to "novelty" in the prohibitions would be deleted.

- D. • It was suggested by EMBO that the list of pathogenic organisms under the Prohibitions, especially those in Class 5, may not be appropriate for all European countries and that such classification should be the responsibility of national or regional authorities. Would the Committee object to a footnote here stating that the prohibition on etiologic agents relates to research in the United States?
- E. • In response to commentators' suggestions, a statement of general applicability, as noted above, would be included in this section. On the basis of extensive public comment (discussed more fully in the following section on Roles and Responsibilities), it is suggested that all institutions receiving any NIH funding for recombinant DNA research shall comply with the NIH Guidelines. I would appreciate the Committee's views on this recommendation.

## II. Containment

### Physical Containment

There were a large number of public comments urging greater detail in the safety practices and procedures. Several commentators advised that Appendix D be retained and expanded rather than deleted. I fully agree with these suggestions. Accordingly, Dr. W. Emmett Barkley has convened a committee that is currently restructuring

Appendix D as a safety manual for local institutions where recombinant DNA research is taking place. Additionally, in view of the large number of recommendations for training courses in safety, the NIH has awarded a contract to the American Society for Microbiology to review and possibly develop standards for training in microbiological techniques for recombinant DNA research.

ISSUES FOR THE COMMITTEE'S CONSIDERATION

F. Several commentators pointed out that the proposed revised Guidelines do not require an autoclave in the P3 laboratory itself, but only within the building. The current Guidelines provide for an autoclave "within the building, and preferably within the controlled laboratory area." In light of the concerns expressed, would the Committee object to retaining the language in the current Guidelines?

Biological Containment

There were a number of comments on the development of alternate host-vector systems, the certification process, and distribution of certified host-vectors. Specific suggestions were also received regarding each of the HV systems. On the basis of those comments, I recommend that the Committee consider the following:

ISSUES FOR THE COMMITTEE'S CONSIDERATION

G. • Concerning the HV1 system, objections were made to a requirement that the host should have "a low potential for survival in its natural environment." Many of the host cells that investigators may wish to use have no natural environment other than the

the laboratory. Would the Committee object to the criteria being modified and the language amended to read as follows: "low potential for survival outside the laboratory"?

- H. ● It was urged that the requirement for independent confirmation of relevant phenotypic and genotypic traits for certification at the HV3 level should also be applied at the HV2 level. In light of this suggestion, the question arises why the confirmation is required at the HV3 level. Is this done to ensure the credibility of the investigator's results or to determine the range of safety? It has been pointed out that the working groups reporting to your Committee who review the data are, in effect, conducting an independent check for the HV2 level. If so, this check would seem to be sufficient, and indeed, a requirement for an independent check at either the second or third levels would be unnecessary. I would appreciate your views on this subject.
- I. ● There have been a number of suggestions that NIH provide a means to accept requests from the private sector to (a) interpret the Guidelines, (b) certify new host-vector systems, and (c) provide for exemptions from the Guidelines. Your comments on the NIH providing such services would be much appreciated. I would be especially interested in your views on the Committee functions for certification of new host-vector systems where proprietary or patent information might be involved. Would the Committee be willing to accept such a

responsibility? It might be noted in the Guidelines that before review by the Recombinant Advisory committee, developers of new host-vector systems should consider filing for patent protection so that the RAC would be free to disclose information.

### III. Experimental Guidelines

#### Prohibited Experiments

For reasons cited previously, the section on Prohibited Experiments has been transposed to Part I. Please see the discussion above.

#### E. Coli K-12 Host-Vector Systems

A number of commentators asked that the rationale for the classification of permissible experiments be clearly explained. Some pointed out that the classification appeared arbitrary, depending on judgment rather than demonstrable fact. It was further suggested that revisions have not brought us closer to establishing absolute hazard levels.

There were also a number of specific comments here and in later sections on the containment requirements set for DNA viruses and DNA transcripts of retroviruses. On the basis of extensive scientific comment before and during the public hearing, a special U.S./EMBO Workshop and subsequent U.S. Working Group were convened to review this research area. On the basis of that meeting, recommendations have been made for provisions that are before you for action.

A number of recommendations were also received from the agricultural community urging further review and revision of the Guidelines for work with plant pathogens. As a result, another workshop was

convened, and recommendations from that group are also before you. In addition to these general recommendations, other specific issues follow for your review:

- J. • Section (g), "cloning of viral genomes from eukaryotic cell DNA" (F.R. 49601), does not give guidance on containment categories for "shotgun" experiments attempting to clone nucleotide sequences of integrated DNA viruses. At present this section only refers to endogenous retrovirus genomes. It is not clear from the Guidelines what the Committee suggests as containment levels for shotgun experiments attempting to clone integrated polyoma or SV40 sequences. I would appreciate your comments.
- K. • It was urged by several commentators that the CDC classification for Class 2 organisms be revised so that it does not include harmless types of bacteria. What are the Committee's views on providing in the Guidelines that the Director of NIH, on recommendation of your Committee, may designate certain of the agents in CDC's Class 2 as Class 1 agents for purposes of the Guidelines?
- L. • A commentator urged that the cloning into E. coli K-12 of "shotguns" of all nonpathogenic bacteria be placed at the P2 + EK1 or P1 + EK2 level without extensive characterization (Federal Register, page 49602, first column). What are the Committee's views on this recommendation which would eliminate the need for characterization and approval by the RAC?
- M. • It was noted that experiments involving many prokaryotes that

exchange genetic information with E. coli are classified under the present Guidelines at the P1 + EK1 level. Under the proposed revisions this category would be eliminated because presumably they would go on the exempted list. If there is a delay in creating exemptions, however, the investigator would be forced to use the higher containment level in the proposed revisions (under the category of prokaryotes that do not exchange genetic information with E. coli). What are the Committee's views on placing in this section a statement to the effect that any experiments with prokaryotic DNA recombinants that do exchange genetic information with E. coli may be conducted under the containment levels of the 1976 Guidelines unless and until they appear on the exempted list?

- N. • Since synthetic DNA will now be explicitly included in the Guidelines, language must be added concerning the proper containment levels. What are the views of the Committee on the following proposed language?

"Synthetic DNAs

"If the synthetic DNA segment can or might yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically disruptive agent), the containment conditions should be the same as would be used for propagating the natural DNA counterpart.

"If the synthetic DNA sequence codes for a harmless prod-

uct, it may be propagated at the same containment level as its purified natural DNA counterpart. For example, a synthetic DNA segment, to be propagated in E. coli K-12, which corresponds to a nonharmful gene of birds, would require P2 physical containment plus and an EK1 host-vector, or P1 + EK2.

"If the synthetic DNA segment is not expressed as a polynucleotide or polypeptide product, or the products have no natural counterparts, the organisms containing the recombinant DNA molecules are exempt from the Guidelines."

0. • The 1976 Guidelines and your proposed revised Guidelines in the section on "Fungal or Similar Lower Eukaryotic Host-Vector Systems" give little detail, "since the development of these host-vectors is presently in the speculative stage." The recent development of a Saccharomyces cerevisiae system makes this obsolete and suggests that this section be expanded. What is the Committee's view on the following proposed language?

"Fungal or Similar Lower Eukaryotic Host-Vector Systems"

"The containment criteria for DNA recombinant experiments using these host-vectors most closely resemble those for prokaryotes, rather than those for the preceding eukaryotes, since the host cells usually exhibit a capacity for dissemination outside the laboratory that is similar to that for bacteria. Therefore, the procedures established for certification of HV systems other than E. coli K-12 (Section II-D-2) will also

apply to these fungal or similar lower eukaryotic host-vector systems.

"Once approved by NIH, HV1 systems may be used under P2 containment for shotgun experiments with phages, plasmids, and DNA from nonpathogenic prokaryotes and lower eukaryotes that do not produce polypeptide toxins (i.e., organisms that can be cloned into EK1 hosts under P2 conditions). Should HV2 or HV3 systems of this type be developed and approved by NIH, guidelines for their use in other types of recombinant DNA experiments will also be established."

#### IV. Roles and Responsibilities

There was a great deal of comment directed to the relevant roles and responsibilities of the institutions and NIH as outlined in this section. Several commentators requested more information and greater clarification of the structure and operation of the institutional bio-hazards committees (to be called "institutional biosafety committees" in the revision), the function of the biological safety officer, and the duties of the institution. Many other comments were devoted to the membership and functions of the RAC and the responsibilities of the Director, NIH, under the proposed revisions. In light of these comments and my review of the administration of the Guidelines over the past two years, I would like the Committee to consider the following suggestions:

that the contents of Part IV be subsumed under three general

headings--namely, Institution, NIH, and Compliance--with responsibilities listed under appropriate subheadings;

- P. ● that application of the Guidelines extend to all recombinant DNA research done at institutions that receive NIH funding for recombinant DNA research;
- Q. ● that all recombinant DNA research at NIH-funded institutions, irrespective of the projects' source of funds, be reviewed and approved by the Institutional Biosafety Committee (IBC);
- R. ● that the authority be delegated from NIH to the IBC for an independent assessment of the safety standards applied, and that Committee approval be sufficient for the research to proceed;
- S. ● that all research protocols reviewed and approved by the IBC be registered with ORDA-NIH and that ORDA would review all actions to ensure compliance with the NIH Guidelines (except for projects under the aegis of other Federal agencies);
- T. ● that the institution assume responsibility for modifying the research protocol as recommended by NIH when NIH has found it in violation of the Guidelines.

In sum, all recombinant DNA research in institutions receiving NIH funding shall be reviewed and approved by the local IBC and registered at NIH or other funding Federal agency. Authority for approval has been delegated to the local biosafety committee. Approvals are subject to subsequent NIH review to ensure compliance with the NIH Guidelines, but investigators will not need approval

from NIH to commence. By this means, national standards will govern, with local responsibility for oversight and monitoring. I would appreciate your views on this extension of the application of the Guidelines with delegation of authority to the local IBCs and appropriate NIH oversight. As noted previously, Appendix D has been substantially revised to provide far greater guidance to the local institution on the evaluation and certification for safe practices and procedures.

The following, for your consideration, summarizes a number of other specific issues that were raised:

Institutional Biosafety Committees

- U. ● It has been suggested that institutional biohazards committees be named "institutional biosafety committees."
- V. ● As noted above, the responsibility of the IBC would be changed to mandate an independent evaluation of the containment levels for the research as required by the Guidelines. (In my 1976 Decision on the Guidelines, I stated that NIH should not require the local institutions to have their committees perform this function, although I did not prohibit them from doing so.)
- W. ● It has been suggested that public membership on the IBC be mandated.

I would welcome the Committee's views on this recommendation.

Biological Safety Officer

- X. ● There were a number of questions concerning the roles and responsibilities of the biological safety officer. It

has been suggested that the officer should cover all levels of recombinant DNA research. What are the Committee's views on recommending that some person or persons be collectively responsible for biosafety monitoring of recombinant DNA research at the P1 through P3 levels and that a special officer be designated with certain responsibilities at the P4 level?

- Y. • It has also been recommended that the biological safety officer be a member of an IBC. What are the Committee's views on this recommendation?

Compliance

- Z. • It has been suggested that a section on penalties be included in Part IV. Would the Committee object to a compliance section that would state that violation of the NIR Guidelines may result in suspension, limitation, or termination of NIH grants or contracts?
- AA. • It has been suggested that information constituting registration of a project with NIH be included in Part IV. Would the Committee object to a registration section that would specify the basic requirements?
- BB. • As noted previously in the section on Biological Containment, there were many requests for NIH to permit voluntary registration and certification by institutions in the private sector. Would the Committee object to such a provision?
- CC. • There were a number of suggestions that general policy be

included on the protection of proprietary information and patent rights. As you know, it is a recent NIH decision that recombinant DNA research inventions developed under DHEW support may be patented by institutions, subject to the condition that licensees will abide by the safety standards of the NIH Guidelines. Would the Committee object to a provision on disclosure of information that would set general policy guidelines in regard to patents?

Exemptions\*\*\*

It must be emphasized that the following exemptions are not meant to apply to experiments described in the previous section as being prohibited.

The following recombinant DNA molecules are exempt from these Guidelines:

- i. Those that are not in organisms or viruses (However, these should be handled with microbiological techniques equivalent to those of a P1 laboratory and should be inactivated\* prior to disposal).
- ii. Those that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent.
- iii. Those in prokaryotic cells where all the DNA segments of the molecules are derived from genomes known to replicate within the cell used to propagate the DNA, though one or more of the segments may be a synthetic equivalent. (This does not include DNA segments only present in such genomes as a result of prior recombinant DNA experiments).
- iv. Those that consist entirely of DNA segments from different species that exchange chromosomal DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such combinations of DNAs will be prepared and periodically revised by the Director, NIH, with the advice of the Recombinant DNA Molecule Program Advisory Committee. Certain classes are exempt as of publication of these Revised Guidelines. The list is in Appendix X.
- v. Other classes of recombinant DNA molecules if the Director, NIH, on the recommendation of the Recombinant DNA Molecule Program Advisory Committee, after appropriate notice and opportunity for public comment, finds that they do not present a significant risk to health or the environment.

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\* Reference to be made at this point to that part of Emmett Barkley's rewrite of what was Appendix D in the 1976 Guidelines which will give detailed information about ways to inactivate DNA.

\*\*prepared with the help of a working group chaired by Dr. John Spizizen, Scripps Research Foundation (list of members is in Attachment III p.2)

RECOMBINANT DNA MOLECULE PROGRAM ADVISORY COMMITTEE

WORKING GROUP

SUMMER HOUSE INN  
7955 LA JOLLA SHORES DRIVE  
LA JOLLA, CALIFORNIA 92037

MARCH 11, 1978  
9:00 A.M.

SPIZIZEN, Dr. John (Chairman)  
Member, Department of Cellular Biology  
Scripps Clinic and Research Foundation  
La Jolla, California 92037  
714, 459-2390 x402

HELINSKI, Dr. Donald R.  
Department of Biology  
University of California, San Diego  
La Jolla, California 92037  
714, 452-3638

CAMPBELL, Dr. Allan  
Department of Biology  
Stanford University  
Stanford, California 94305  
415, 497-2413

HORNICK, Dr. Richard B.  
Professor and Director  
Division of Infectious Diseases  
School of Medicine  
University of Maryland  
Baltimore, Maryland 21201  
301, 528-7562

FALKOW, Dr. Stanley  
Department of Microbiology  
School of Medicine  
University of Washington  
Seattle, Washington 98105  
206, 543-1444

ZAITLIN, Dr. Milton  
Professor  
Department of Plant Pathology  
Cornell University  
Ithaca, New York 14853  
607, 256-3105

Attachment IV-A

The following organisms of the Enterobacteriaceae family exhibit chromosomal DNA relatedness (20% or more homology of DNA of various pairs tested) and genetic recombination or R-prime (R plasmid carrying chromosomal genes) transfer to E. coli K-12 mediated by the IncP-1 plasmids.

All species of the following genera:

1. Escherichia (including E. coli K-12)
2. Shigellae
3. Salmonella
4. Enterobacter
5. Arizona
6. Citrobacter
7. Klebsiellae

In addition the following species:

1. Erwinia amylovora
2. Erwinia dissolvens
3. Erwinia minipressuralis
4. Serratia marcescens
5. Levinea malonatica
6. Levinea amalonatica

Attachment IV-B

The following organisms exhibit R-prime (R plasmid carrying chromosomal genes) transfer to E. coli K-12 mediated by the IncP-1 plasmids.

1. All members of the Enterobacteriaceae family
2. Vibrio species (excluding Vibrio parahemolyticus)
3. Pseudomonas species
4. Rhizobium species
5. Acinetobacter calcoaceticus
6. Agrobacterium tumefaciens
7. Rhodopseudomonas sphaeroides
8. Caulobacter crescentis

Attachment IV-C

The following genera and/or species possess R plasmids (including R plasmids of the IncP-1 group) transferable to E. coli K-12.

1. All members of the Enterobacteriaceae family
2. Vibrio species (except Vibrio parahemolyticus)
3. Pseudomonas species
4. Rhizobium species
5. Acinetobacter calcoaceticus
6. Agrobacterium tumefaciens
7. Rhodopseudomonas sphaeroides
8. Caulobacter crescentis
9. Proteus species
10. Achromobacter species
11. Aeromonas salmonicida
12. Alcaligenes faecalis
13. Bordetella bronchiseptica
14. Myxococcus xanthus
15. Neisseria gonorrhoeae
16. Pastuerella hemolytica
17. Pastuerella multocida
18. Yersinia species (excludes Y. pestis, since it is a Class 3 agent)
19. Xanthomonas species

In addition, recombinant DNA experiments between H. influenzae, and H. parainfluenzae are exempt on the basis of extensive DNA homology.

Exemptions

It must be emphasized that the following exemptions are not meant to apply to experiments described in the previous section as being prohibited.

The following recombinant DNA molecules are exempt from these Guidelines:

- i. Those that are not in organisms or viruses (See Appendix D for information on inactivating DNA).
- ii. Those that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent.
- iii. Those that are: a) derived from plasmids or viruses indigenous to a bacterial host (or found in nature in that host) where the recombinant DNA molecules prepared from those plasmids or viruses and host genome are propagated within the host (or a closely related member of the same species); or b) derived entirely from the organelles (e.g. chloroplasts or mitochondria) of an organism where the recombinant DNA molecules prepared from the organelles are propagated within that organism (or a closely related member of the same species).

- iv. Those that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such combinations of DNAs will be prepared and periodically revised by the Director, NIH, with the advice of the Recombinant DNA Molecule Program Advisory Committee. Certain classes are exempt as of publication of these Revised Guidelines. The list is in Appendix \_\_\_\_.
  
- v. Other classes of recombinant DNA molecules if the Director, NIH, on the recommendation of the Recombinant DNA Molecule Program Advisory Committee, after appropriate notice and opportunity for public comment, finds that they do not present a significant risk to health or the environment. A list of such will be prepared and periodically revised. Certain classes are exempt as of publication of these Revised Guidelines. The list is in Appendix \_\_\_\_\_.

## Attachment VI

### Proposed Rules for In Vitro Packaging in HV2 Systems Using $\lambda$ Vectors

- (1) The packaging extract must be free from viable bacteria.
- (2) Any packaging protocol may be used, provided that control experiments on the packaging of EK2 vector DNA meet one of the following two criteria:
  - (a) The number of amber<sup>+</sup> phages produced must be less than  $10^{-6}$  times the number of amber<sup>-</sup> phages. If shotgun populations are to be propagated in bulk culture or by confluent lysis methods, this measurement must be made on packaged EK2 vector DNA propagated to the same extent.
  - (b) If the total number of amber<sup>-</sup> phages produced in a packaging experiment is less than  $10^6$ , and if the shotgun population is not to be propagated in bulk, the number of observed amber<sup>+</sup> plaques must be zero.
- (3) The above tests must be done on each batch of packaging extract used.
- (4) Any individual clone isolated from the shotgun must be tested for retention of the safety characteristics of the vector before it can be used for bulk propagation.
- (5) A description of the packaging protocol should be filed with NIH for information, but NIH approval is not required provided that the above numerical criteria are met.

### VERIFICATION OF SAFETY FEATURES IN EK2 VECTORS BEARING CLONED SEGMENTS

Before extensive propagation of an EK2 vector bearing a cloned segment, the investigator must check that the safety features of the vector are unaltered. What specific tests should be performed will vary with the nature of the vector. However, such tests must not include genetic crosses that might produce recombinants in which a vector bearing a cloned segment is disarmed for any of its safety features. Such crosses can only be performed under conditions of physical containment appropriate to an EK1 vector bearing the cloned segment in question.

For example, if the vector is a  $\lambda$  phage bearing two or more amber mutations, its multiple amber character should be checked by plating on a suppressor-minus host, thus verifying that its reversion frequency to amber<sup>+</sup> is less than that expected for a single amber. It should not be crossed with single amber mutants of  $\lambda$  to verify the presence of specific amber mutations.

VALEDICTORY <sup>1/</sup>

by

DeWitt Stetten, Jr. <sup>2/</sup>

I am taking a Chairman's prerogative to invade the printed agenda. I should like at this time to share with you the reasons why I have felt impelled to resign my chairmanship of this Committee. Shortly after our last meeting of November 1977, I asked the Director, NIH, to accept my resignation and find a replacement for this chairmanship. He asked me to assist in the selection of a new Chairman and I have provided to him the names of candidates from which he is soon to make a choice. I am certain that you will be pleased with the name of my successor, and that the Committee will give the new Chairman the same devotion and industry which it has given to me.

There were, of course, personal reasons for my resignation. I am four years older than I was when I was first appointed, I fatigue more easily, and, as you are all aware, my visual acuity has continued to decrease until I am able to read only a very small fraction of the large amount of paper which passes over my desk in relation to this function. In addition, I have had a growing unhappiness with some of the directions which the recombinant DNA program has taken over the past four years. From my conversations with members of the Committee, I believe that this unhappiness is shared by some of you, and this may be a good opportunity to verbalize this discontent.

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<sup>1/</sup> Given at the meeting of the Recombinant DNA Molecule Program Advisory Committee, April 28, 1978, NIH, Bethesda, Maryland

<sup>2/</sup> Deputy Director for Science, National Institutes of Health

Prior to the Asilomar meeting of February 1975, I had had only modest contact with nucleic acids and with genetics. I had worked in the laboratory with lipids, polysaccharides, and proteins, but had never handled any nucleic acids. I had never worked on a genetic problem, and had certainly never engaged in microbiological research. Except for some briefing which I secured from members of the intramural NIH family, I came to Asilomar cold.

It has taken me several years to analyze and unscramble the experience of the Asilomar meeting. I now understand it more fully than I did at the time. It had many elements of a religious revival meeting. I heard several colleagues declaim against sin, I heard others admit to having sinned, and there was a general feeling that we should all go forth and sin no more. The imagery which was presented was surely vivid, but the data were scanty. I recall one scientist presenting information on the difficulty of colonizing the intestinal tract with E. coli K-12, but his presentation was given little attention. We were all, in effect, led down to the river to be baptized and we all went willingly. I, for one, left the meeting enthralled. I had never been to a scientific meeting which had so excited me. On my return to Bethesda, I was asked to summarize the events at Asilomar before a meeting of the generally staid NIH Institute Directors and I believe I was able to transfer to them some of my excitement. Over the succeeding months, the Recombinant DNA Molecule Program Advisory Committee met and, by July 1975, it drafted a set of guidelines at Woods Hole, Massachusetts, which I at the time thought to be reasonably satisfactory. They did not conform to my prior notion of guidelines exactly, since they bordered on

the encyclopedic. Nonetheless, I felt that we had successfully compromised most of the burning issues over which the Committee was initially strongly divided. When these guidelines were distributed, however, they elicited vigorous and often emotional responses, and among these responses there was one which I recall vividly. It charged our Committee with having violated the "spirit of Asilomar." At the time this expression did not catch my attention, but on consideration I was struck by the fact that despite the many, many meetings which I had attended at Atlantic City, I had never heard a reference to the "spirit of Atlantic City." This charge, in fact, pinpointed for me the notion that the experience at Asilomar was essentially a spiritual one rather than an intellectual one. It was, in the usual sense, not a scientific meeting at all. Whatever its purpose may have been in the minds of its initiators, a result was to fire the imagination, first, of the newspaper correspondents who were abundantly represented, and then of a substantial segment of the newspaper-reading public.

By December 1975, our Committee, meeting at La Jolla, again assembled a set of guidelines. Whereas up to that time I had insufficient confidence in my own judgment to hold a firm opinion on this issue, and found myself swayed by the views most recently presented, it was about the time of the La Jolla meeting that I began to wonder whether, indeed, any of the postulated hazards of recombinant DNA molecule technology were likely to materialize.

The La Jolla guidelines served as the basis for a discussion at a meeting of the NIH Director's Advisory Committee early in 1976, and this, in turn, was followed in July by the publication of the official NIH

guidelines. In this last transformation, something happened which I found disturbing.

The mission of NIH is, I believe, very simply stated. It is to conduct and to support the very best biomedical research that it can find to conduct and support. Similarly, the mission of our Committee and of the guidelines which it drafted was to provide assurance that research in the area of recombinant DNA molecules would be conducted in such a fashion as not to jeopardize the laboratory, the community, or the environment. Both missions, it should be noted, are stated positively. It is the purpose both of NIH and of this Committee to encourage, to promote--not to forbid or to impede. The legal profession represented at the Director's Advisory Committee meeting was critical of the concept of guidelines, which in my judgment are designed to provide guidance to the investigator and to those who review his proposal. We were informed that what was needed was regulation, not guidance. This was exemplified by the recommendation that our instruction, written largely in the subjunctive mood (the investigator should . . .) be replaced by the more peremptory language of regulations (the investigator shall . . .). I recall arguing against such change in vain.

My reasons were very simple. It is my interpretation of the history of science and indeed of all culture that regulation is antithetical to creativity, and creativity is the most important component of scientific advance. From this, it follows that the best regulation for the flowering of science is the least regulation--that is, the least regulation compatible with the needs of society. Furthermore, I feared and my fears were, I think,

justified that regulation might lead to legislation with a specification of sanctions, i.e., punishment, for those who were in violation of the regulations. Whereas the so-called regulatory agencies of Government must from time to time adopt a punitive posture, this is, I believe, a poor posture for a research agency such as the National Institutes of Health.

Against what hazards were we proposing to draft regulations? With the passage of time, the hazards that had been pictured at Asilomar seemed to recede. Whereas a great number of positive and useful scientific results are being published based upon the technology of recombinant DNA molecules, to the best of my knowledge no adverse results have been noted. Indeed, I believe that there is at this time not one iota of acceptable evidence, i.e., data publishable in a scientific journal, to indicate that the recombinant DNA molecule technology has ever enhanced the pathogenicity or the toxigenicity of any microorganism. This, of course, does not mean that it never will do so, but it does cause one to wonder whether all of the present fuss is truly justified. It places the hazards in this area in the same category as those in many other areas for which we have no positive evidence. To clarify this point, let me offer you an analogy. Ever since the Middle Ages, it has been suspected that the ghosts of those who died by suicide are more menacing than ghosts in general. This anxiety, once implanted in the minds of the people, led to some interesting containment practices. The bodies of victims of suicide were excluded from traditional burial places, lest their ghosts pollute or otherwise disturb the more peaceful ghosts of those who died of natural causes. They were doomed to be buried in the crossroads, and in order to ensure that the ghosts not

escape from the tomb, a stake was driven through the body of the victim into the underlying soil, thus pinning the ghost into its grave. This containment practice continued for many centuries and was ultimately abandoned only in the 18th century. Experience since that time has justified the conclusion--that the hazard which had earlier been postulated was either of very small magnitude or possibly nonexistent. We may yet prove to be wrong about the safety of unpinning the ghosts of suicide victims, but I should be surprised if this were so.

How long do we wait, in the absence of any positive evidence, before we decide that the hazards in a particular area of research are at a socially acceptable level? To this question I have no specific answer. Soon we may come to the conclusion that the manipulations of recombinant DNA technology do not of themselves add significantly to the dangers inherent in the conduct of microbiological research. Then we can replace our complex and, I repeat, encyclopedic guidelines by a very simple statement. This might take the following form: "The conditions of containment appropriate for any recombinant DNA experiment are those which are dictated by the most virulent or dangerous organism entering into that experiment." Is anything more really required?

I hope that none of you will construe any of my critical remarks as being personally directed. They are not. I have thoroughly enjoyed and been stimulated by my contacts with the many members of the Committee. I hope that I have established enduring friendships with many of you, and I shall certainly follow your further deliberations with great interest and concern. I should like particularly to express my appreciation to the

several members of the NIH staff who have worked so hard and so loyally to keep this project afloat: Dr. Leon Jacobs who, from the beginning, has served as Co-Chairman of this Committee, Dr. Bernard Talbot, who has worked enormously hard and valiantly, Dr. William Gartland, Director of the Office of Recombinant DNA Activities, his small but energetic staff-- Dr. Kamely and Dr. Goldberg. Then, there is Ms. Betty Butler, who not only made certain that all the paper flowed in the right directions but also nursed us through our several tortured meetings. To work with all of these people has been a very rewarding experience.

I wish you well in your future meetings.

Table 1

Recommended Containment for Cloning of Viral DNA or cDNA in *E. coli* K12 Host-Vector Systems  
(See Text for Full Details)

Virus Class	Type of Viral DNA Segment to be Cloned				cDNA from <sub>3</sub> viral mRNA <sup>3</sup>
	Subgenomic <sup>1</sup>		Genomic		
	Non-transforming segments	Segments containing an entire transforming gene	Non-segmented genome	Segmented genome	
<u>DNA</u>					
Non-transforming viruses					
AAV, MVM, Mouse Adeno Strain FL	P1 <sup>4</sup> +EK1		P1 <sup>4</sup> +EK1		P1 <sup>4</sup> +EK1
Plant Viruses	P1 <sup>4</sup> +EK1		P1 <sup>4</sup> +EK1		P1 <sup>4</sup> +EK1
Other Presently Classified Viruses <sup>7</sup>	P1 <sup>4</sup> +EK1		P1 <sup>4</sup> +EK1CV <sup>5</sup>		P1 <sup>4</sup> +EK1
Transforming Viruses					
Herpes saimiri and H. ateles <sup>6</sup>	P1 <sup>4</sup> +EK1	P2+EK1CV <sup>5</sup>	P2+EK2 or P3+EK1		P2+EK1CV <sup>5</sup>
Other Presently Classified Viruses <sup>7</sup>	P1 <sup>4</sup> +EK1	P2+EK1CV <sup>5</sup>	P2+EK1CV <sup>5</sup>		P2+EK1CV <sup>5</sup>
<u>RNA</u>					
Retroviruses					
Gibbon Ape and Woolly Monkey <sup>6</sup>	P1 <sup>4</sup> +EK1	P2+EK1CV <sup>5</sup>	P2+EK2 or P3+EK1		P2+EK1
Other Presently Classified Viruses <sup>7</sup>	P1 <sup>4</sup> +EK1	P2+EK1CV <sup>5</sup>	P2+EK1CV <sup>5</sup>		P2+EK1
Negative Strand RNA	P1 <sup>4</sup> +EK1		P1 <sup>4</sup> +EK1	P1 <sup>4</sup> +EK1	P1 <sup>4</sup> +EK1
Plus Strand RNA					
Type 1 and 2 Sabin Polio, 17D					
Yellow Fever Vaccine Strains	P1 <sup>4</sup> +EK1		P1 <sup>4</sup> +EK1		P1 <sup>4</sup> +EK1
Other Presently Classified Viruses <sup>7</sup>	P1 <sup>4</sup> +EK1		P2+EK1CV <sup>5</sup>		P2+EK1CV <sup>5</sup>
Double Stranded RNA	P1 <sup>4</sup> +EK1			P1 <sup>4</sup> +EK1	P1 <sup>4</sup> +EK1
Plant Viruses + Viroids	P1 <sup>4</sup> +EK1		P1 <sup>4</sup> +EK1	P1 <sup>4</sup> +EK1	P1 <sup>4</sup> +EK1
<u>Intracellular Viral DNA</u> <sup>2</sup>	See text	See text	See text		

- <sup>1</sup>  $\geq 99\%$  pure (i.e., less than 1% of the DNA consists of intact viral genomes); otherwise as for whole genomes.
- <sup>2</sup> Integrated genomes to be cloned at containment as for shotgun experiments with eukaryotic cellular DNA.
- <sup>3</sup> The cDNA copy of viral mRNA must be  $\geq 99\%$  pure; otherwise as for shotgun experiments with eukaryotic cellular DNA.
- <sup>4</sup> For all these experiments, P1 is defined as including a ban on mouth pipetting.
- <sup>5</sup> EK1CV means the use of an EK1 host and a vector certified for use in an EK2 system.
- <sup>6</sup> These viruses have been classified by NCI as "moderate risk oncogenic viruses," and NCI recommends that the viruses themselves be handled under the equivalent of P3 containment.
- <sup>7</sup> As classified in the Second Report of the International Committee on Taxonomy of Viruses: Classification and Nomenclature of Viruses, Frank Fenner, Ed. Intervirology 7 (19-115) 1976. (As noted in the Prohibition Section, the use of viruses classified as Class 3, 4, or 5, in the CDC Classification of Etiologic Agents on the Basis of Hazard, other than VSV, is prohibited.)

(1) Viruses of Eukaryotes

(a) DNA Viruses

1. Non-transforming viruses

(a) Adeno-associated viruses, minute virus of mice, mouse adenovirus strain FL and plant viruses - P1 physical

containment including no mouth pipetting + an EK1

host-vector shall be used for DNA recombinants produced

with: (a) the whole viral genome; (b) subgenomic DNA segments; or (c) cDNA copies of viral mRNA<sup>3</sup>.

(b) Other presently classified viruses<sup>7</sup>

(i) P1 physical containment including no mouth pipetting + an EK1 host-vector shall be used for: (a) DNA recombinants produced with purified subgenomic DNA segments<sup>1</sup>; or (b) cDNA copies of viral mRNA<sup>3</sup>.

(ii) P1 physical containment including no mouth pipetting + an EK1 host and a vector certified for use in an EK2 system shall be used for DNA recombinants produced with the whole viral genome.

2. Transforming viruses

(a) Herpes saimiri and Herpes ateles<sup>6</sup>

(i) P1 physical containment including no mouth pipetting + an EK1 host-vector shall be used for DNA recombinants produced with purified non-transforming subgenomic DNA segments.

(Note: Footnotes 1-7 used in this attachment and Table 1 are defined at the bottom of Table 1. When this material is integrated into the Guidelines, the footnote numbering will have to be changed.)

- (ii) P2 physical containment + an EK1 host and a vector certified for use in an EK2 system shall be used for: (a) DNA recombinants produced with purified subgenomic DNA segments<sup>1</sup> containing an entire transforming gene; or (b) cDNA copies of viral mRNA<sup>3</sup>.
- (iii) P3 physical containment + an EK1 host-vector or P2 + EK2 shall be used for DNA recombinants produced with the whole viral genome.

(b) Other presently classified viruses

- (i) P1 physical containment including no mouth pipetting + an EK1 host-vector shall be used for DNA recombinants produced with purified non-transforming subgenomic DNA segments<sup>1</sup>.
- (ii) P2 physical containment + an EK1 host and a vector certified for use in an EK2 system shall be used for: (a) DNA recombinants produced with the whole viral genome; (b) purified subgenomic DNA segments containing an entire transforming gene; or (c) cDNA copies of viral mRNA<sup>3</sup>.

(b) RNA Viruses

1. Retroviruses

(a) Gibbon ape and Woolly monkey viruses<sup>6</sup>

- (i) P1 physical containment including no mouth pipetting + an EK1 host-vector shall be used for DNA recombinants produced with purified non-transforming subgenomic DNA segments<sup>1</sup>.

- (ii) P2 physical containment + an EK1 host and a vector certified for use in an EK2 system shall be used for DNA recombinants produced with: (a) purified sub-genomic DNA segments<sup>1</sup> containing an entire transforming gene; or (b) cDNA copies of viral mRNA<sup>3</sup>.
  - (iii) P2 physical containment + an EK2 host-vector or P3 + EK1 shall be used for DNA recombinants produced with the whole viral genome.
- (b) Other presently classified viruses<sup>7</sup>
- (i) P1 physical containment including no mouth pipetting + an EK1 host-vector shall be used for DNA recombinants produced with purified non-transforming subgenomic DNA segments<sup>1</sup>.
  - (ii) P2 physical containment + an EK1 host and a vector certified for use in an EK2 system shall be used for DNA recombinants produced with: (a) purified sub-genomic DNA segments containing an entire transforming gene; (b) the whole viral genome; or (c) cDNA copies of viral mRNA<sup>3</sup>.
2. Negative strand RNA viruses - P1 physical containment including no mouth pipetting + an EK1 host-vector shall be used for DNA recombinants produced with: (a) the whole genome; (b) subgenomic DNA segments; or (c) purified cDNA copies of viral mRNA<sup>3</sup>.
3. Plus-strand RNA viruses
- (a) Types 1 and 2 Sabin poliovirus vaccine strains and strain 17D (Theiler) of yellow fever virus - P1 physical containment

including no mouth pipetting + an EK1 host-vector shall be used for DNA recombinants produced with: (a) the whole viral genome; (b) subgenomic DNA segments; or (c) purified cDNA copies of viral mRNA<sup>3</sup>.

(b) Other presently classified viruses<sup>7</sup>

(i) P1 physical containment including no mouth pipetting + an EK1 host-vector shall be used for DNA recombinants produced with purified subgenomic DNA segments<sup>1</sup>.

(ii) P2 physical containment + an EK1 host and a vector certified for use in an EK2 system shall be used for DNA recombinants produced with: (a) the whole genome; or (b) purified cDNA copies of viral mRNA<sup>3</sup>.

4. Double-stranded segmented RNA Viruses - P1 physical containment including no mouth pipetting + an EK1 host-vector shall be used for DNA recombinants produced with: (a) mixtures of subgenomic DNA segments; (b) a specific subgenomic DNA segments; or (c) purified cDNA copies of viral mRNA<sup>3</sup>.

5. All Plant Viruses and Plant Viroids - P1 physical containment including no mouth pipetting + an EK1 host-vector shall be used for DNA recombinants produced with: (a) the whole viral genome; (b) subgenomic DNA segments; or (c) cDNA copies of viral mRNA<sup>3</sup>.

(c) Intracellular Viral DNA - Physical and biological containment specified for shotgun experiments with eukaryotic cellular DNA (Sec. III-B-1-a-(1)) shall be used for DNA recombinants produced with integrated viral DNA or viral genomes present in infected cells.

Table 2  
Recommended Containment for Recombinant DNA Research Using Eukaryotic Viral Vectors  
(See Text for Full Details)

Vector DNA	Productive Virus-Cell Interactions					Eukaryotic Viral	Non-Productive Virus-Cell Interactions
	Type of DNA Insert						
	Prokaryotic		Eukaryotic		Purified <sup>9</sup>		
	Shotgun	Purified	Shotgun				
Natural Host			Other				
1. Polyoma							
Intact Genome	P2	P2	P2	P3	P2	CBC*	P2 <sup>8</sup>
Deleted Genome	P2	P2	P2	P2	P2	CBC*	P2 <sup>8</sup>
2. SV40							
Intact Genome	--	--	--	--	--	---	P2 <sup>8</sup>
Deleted Genome	P2	P2	P2	P3	P3	CBC*	P2 <sup>8</sup>
3. Human Ad2+Ad5							
Deleted Genome	P3	P3	P3	P3	P3	CBC*	P2 <sup>8</sup>
4. Mouse Adenovirus (Strain FL)							
Intact Genome	CBC*	CBC*	CBC*	CBC*	CBC*	CBC*	P2 <sup>8</sup>
Deleted Genome	P2	P2	P2	P2	P2	CBC*	P2 <sup>8</sup>
5. Insect Viruses	CBC*	CBC*	CBC*	CBC*	CBC*	CBC*	--
6. Plant Viruses (CaMV and BGMV)	**	**	**	**	**	CBC*	--
7. All other potential Viral Vectors	CBC*	CBC*	CBC*	CBC*	CBC*	CBC*	CBC*

\* CBC - Case by case

\*\*See Text

<sup>8</sup> Provided the inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated on a case-by-case basis.

<sup>9</sup> ≥99% pure; otherwise as for shotgun experiments.

3. Experiments with Eukaryotic Host-Vectors

a. Vertebrate Host-Vector Systems

Because this work will be done almost exclusively in tissue culture cells, which have no capacity for propagation outside the laboratory, the primary focus for containment is the vector; it should be pointed out that risk of laboratory acquired infection as a consequence of tissue culture manipulations is very low. Given good microbiological practices, the most likely mode of escape of recombinant DNAs from a physically contained laboratory is carriage by an infected human; thus the vector with an inserted DNA segment should have little or no ability to replicate or spread in humans. Further, a recombinant virus should not inadvertently pose a threat to any species.

For use as a vector in a vertebrate host cell system, an animal viral DNA molecule should display the following properties:

- (a) It should not consist of the whole genome of any agent that is infectious for humans or that replicates to a significant extent in human cells in tissue culture. If the recombinant molecule is used to transform non-permissive cells (i.e. cells which do not produce infectious virus particles), this is not a requirement.
- (b) It should be derived from a virus whose epidemiological behavior and host range are well understood.
- (c) In permissive cells, it should be defective when carrying an inserted DNA segment; (i.e. propagation of the recombinant DNA as a virus must be dependent upon the presence of a complementing helper genome). In almost all cases this condition would be achieved automatically by the manipulations used to construct

and propagate the recombinants. In addition, the amount of DNA encapsidated in the particles of most animal viruses is defined within fairly close limits. The insertion of sizeable foreign DNA sequences, therefore, generally demands a compensatory deletion of viral sequences. It may be possible to introduce very short insertions (50-100 base pairs) without rendering the viral vector defective. In such a situation, the requirement that the viral vector be defective is not necessary except in those cases in which the inserted DNA encodes a biologically active polypeptide.

It is desired but not required that the functional anatomy of the vector be known -- that is, there should be a clear idea of the location within the molecule of:

- (a) The sites at which DNA synthesis originates and terminates.
- (b) The sites that are cleaved by restriction endonucleases.
- (c) The template regions for the major gene products.

If possible the helper virus genome should:

- (i) be integrated into the genome of a stable line of host cells (a situation that would effectively limit the growth of the vector recombinant to such cell lines) or
- (ii) consist of a defective genome, or an appropriate conditional lethal mutant virus, making vector and helper dependent upon each other for propagation.

However, neither of these stipulations is a requirement.

(1) Polyoma Virus

- (a) Productive virus-cell interactions

1. Defective or intact polyoma virus genomes, with appropriate helper, if necessary, can be used in P2 conditions to propagate DNA sequences from:
    - (a) bacteria of CDC class 1 or class 2 (see Appendix B), or their phages or plasmids, except for those that produce potent polypeptide toxins.
    - (b) from mice
    - (c) from other eukaryotic organisms that do not produce potent polypeptide toxins, provided the DNA segment is  $\geq 99\%$  pure.
  2. Defective polyoma genomes, with appropriate helper, if necessary, can be used in P2 conditions for shotgun experiments to propagate DNA sequences from eukaryotic organisms that do not produce potent polypeptide toxins.
  3. Intact virus genomes with appropriate helper, if necessary, can be used in P3 conditions for shotgun experiments to propagate DNA sequences from eukaryotic organisms that do not produce potent polypeptide toxins.
  4. Experiments involving the use of defective polyoma virus genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by the NIH on a case-by-case basis and will be conducted under the recommended physical containment conditions.
- (b) Non-productive virus-cell interactions - Defective or intact polyoma virus genomes can be used as vectors in P2 conditions to transform non-permissive cells in culture provided the inserted DNA sequences are not derived from eukaryotic viruses.

In the latter case, such experiments will be evaluated by NIH on a case-by-case basis.

(2) Simian Virus 40

(a) Productive virus-cell interactions

1. SV40 DNA, rendered unconditionally defective by a deletion in an essential gene, with appropriate helper, can be used in P2 conditions to propagate DNA sequences from:
  - (a) bacteria of CDC Class 1 or Class 2 (see Appendix B), or their phages or plasmids, except for those that produce potent polypeptide toxins.
  - (b) uninfected African green monkey kidney cell cultures.
2. SV40 DNA, rendered unconditionally defective by a deletion in an essential gene, with an appropriate helper, can be used in P3 conditions to propagate DNA sequences from eukaryotic organisms that do not produce potent polypeptide toxins (shotgun experiments or  $\geq$  99% purified DNA).
3. Experiments involving the use of defective SV40 genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by the NIH on a case-by-case basis and will be conducted under the recommended physical containment conditions.

- (b) Non-productive virus-cell interactions - Defective or intact SV40 genomes can be used as vectors in P2 conditions to transform non-permissive cells in culture provided the inserted DNA sequences are not derived from eukaryotic viruses.

In the latter case, such experiments will be evaluated by NIH on a case-by-case basis.

(3) Human Adenoviruses 2 and 5

(a) Productive virus-cell interactions

1. Human adenoviruses 2 and 5, rendered unconditionally defective by deletion of at least 2 capsid genes, with appropriate helper(s), can be used in P3 conditions to propagate DNA sequences from:

(a) bacteria of CDC Class 1 or Class 2 (see Appendix B) or their phages or plasmids except for those that produce potent polypeptide toxins.

(b) eukaryotic organisms that do not produce potent polypeptide toxins (shotgun experiments or  $\geq$  99% purified DNA).

2. Experiments involving the use of unconditionally defective human Ad 2 and 5 genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by the NIH on a case-by-case basis and will be conducted under the recommended physical containment conditions.

(b) Non-productive virus-cell interactions - Defective or intact

human Ad 2 and 5 genomes can be used as vectors in P2 conditions to transform non-permissive cells in culture provided the inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis.

(4) Murine Adenovirus Strain FL

(a) Productive virus-cell interactions

1. Unconditionally defective murine adenovirus strain FL genomes, with appropriate helper, can be used in P2 conditions to propagate DNA sequences from:
  - (a) bacteria of CDC Class 1 or Class 2 (see Appendix B) or their phages or plasmids except for those that produce potent polypeptide toxins.
  - (b) eukaryotic organisms that do not produce potent polypeptide toxins (shotgun experiments or  $\geq 99\%$  purified DNA).
2. Experiments involving the use of intact murine adenovirus strain FL genomes to propagate DNA sequences from prokaryotic or eukaryotic organisms will be evaluated by the NIH on a case-by-case basis and will be conducted under the recommended physical containment conditions.
3. Experiments involving the use of unconditionally defective murine adenovirus strain FL genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by the NIH on a case-by-case basis and will be conducted under the recommended physical containment conditions.

- (b) Non-productive virus-cell interactions - Defective or intact murine adenovirus strain FL genomes can be used as vectors in P2 conditions to transform non-permissive

cells in culture provided the inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis.

(5) All other potential viral vectors

(a) Experiments involving the use of viral DNA vectors consisting of 25% or less of the virus genome can be done:

1. in P2 conditions to transform cells in culture provided the inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis.
2. under physical containment conditions to be determined by the NIH to propagate DNA sequences from prokaryotic or eukaryotic organisms, with an appropriate helper.

(b) Experiments involving the use of other intact or defective virus genomes to propagate DNA sequences from prokaryotic or eukaryotic organisms (and viruses) or as vectors to transform non-permissive cells will be evaluated by the NIH on a case-by-case basis and will be conducted under the recommended physical containment conditions.

The NIH will also review all experiments involving the use of virus vectors in animals and the physical containment conditions appropriate for such studies.

b. Invertebrate Host-Vector Systems in which Insect Viruses are used to Propagate other DNA Segments

As soon as information concerning the nature of the host range, infectivity, persistence and integration in vertebrate and invertebrate cells becomes available, experiments involving the use of insect viruses to propagate DNA sequences will be evaluated by the NIH on a case-by-case basis and will be conducted under the recommended physical containment conditions. Experiments should be done in established invertebrate cell lines and should follow, where appropriate, criteria recommended for vertebrate viral DNA vectors [Sec. III-B-3-a/].

c. Plant Viral Host-Vector Systems

The DNA plant viruses which could currently serve as vectors for cloning genes in plants and plant cell protoplasts are Cauliflower Mosaic Virus (CaMV) and its close relatives, which have relaxed circular double stranded DNA genomes with a molecular weight of  $4.5 \times 10^6$ , and Bean Golden Mosaic Virus (BGMV) and related viruses with small ( $<10^6$  daltons) single-stranded DNA genomes. These viruses are not known to integrate into host chromosomes, or to incorporate cellular genes into their genomes. CaMV is spread in nature by aphids, in which it survives for a few hours. Spontaneous mutants of CaMV that are not transmitted by aphids arise frequently; these mutants fail to make a transmission factor essential for aphid transmission. BGMV is spread in nature by whiteflies, in which it survives for several days to three weeks; certain other single-stranded DNA plant viruses are transmitted by leafhoppers, in which the viruses persist for days or weeks. Single-stranded DNA plant viruses are thought not to replicate in their insect vector.

The DNA plant viruses have narrow host ranges and are relatively difficult to transmit mechanically to plants. For this reason, they are most unlikely to be accidentally transmitted from spillage of purified preparations of the virus.

When these viruses are used as vectors in intact plants, or propagative plant parts, the plants should be grown under PI conditions, that is in either a limited access greenhouse or plant growth cabinet which is insect-proof, preferably with positive air pressure, and in which an insect fumigation regime is maintained. Soil, plant pots and unwanted infected plant materials should be removed from the greenhouse or cabinet in sealed insect proof

containers and sterilized. It is not necessary to sterilize run-off water from the infected plants as this is not a plausible route for secondary infection. When the viruses are used as vectors in tissue cultures or in small plants in axenic cultures, no special containment is recommended. Infected plant materials to be used for further research, which have to be removed from the greenhouse or cabinet, should be maintained under insect proof conditions. These measures provide an entirely adequate degree of containment and are similar to those required in many countries for licensed handling of "exotic" plant viruses.

The viruses or their DNA may also be useful as a vector to introduce genes into plant protoplasts. The fragility of plant protoplasts combined with the properties of the viruses mentioned above provide adequate safety. Since no risk to the environment from the use of the DNA plant virus/protoplast system is envisaged, no special containment is recommended, except as described in the following paragraph.

Experiments involving the use of plant virus genomes to propagate DNA sequences from eukaryotic viruses will be evaluated on a case-by-case basis and will be conducted under the recommended containment conditions.

HAZARD CLASSIFICATION OF PLANT PATHOGENS

Class 1A Plant pathogens not in Class 1B.

Class 1B All organisms that are subject to quarantine restrictions for any of the following reasons:

- (i) Plant pathogens not known to occur in the U.S.
- (ii) Plant pathogens that are not widely distributed throughout the ecological range of their hosts.
- (iii) Plant pathogens subject to federal or state eradication or suppression programs.

All plant pathogens whether domestic or foreign require state and federal (USDA<sup>1</sup>) permits for shipment across state lines.

<sup>1</sup>Address to obtain application to import or move a plant pest or pathogen: Plant Importation and Technical Support Staff, Plant protection and Quarantine Programs, Animal and Plant Health Inspection Service, USDA, Federal Center Building, Hyattsville, Md. 20782.