

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

RECOMBINANT DNA ADVISORY COMMITTEE  
WORKING GROUP ON REVISION OF THE GUIDELINES

MINUTES OF MEETING

JANUARY 21, 1983

The Working Group on Revision of the Guidelines was convened at 9:00 a.m. on January 21, 1983, in Building 31, Room 7A24, at the National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20205. The meeting was open to the public. Dr. Elena Nightingale was chairperson.

Working Group members present for all or part of the meeting were:

Susan Gottesman, NIH; Malcolm Martin, NIH; John Scandalios, North Carolina State University; Sue Tolin, Department of Agriculture; and Elizabeth Milewski, NIH (Executive Secretary).

Other NIH staff present were:

William Gartland, NIH.

Call to Order and Opening Remarks.

Dr. Nightingale, chairperson, called the meeting to order at 9:10 a.m. She said the agenda would cover several topics:

- (1) agricultural concerns, e.g., what constitutes dissemination into the environment for plants;
- (2) a review of the letters received from Institutional Biosafety Committee (IBC) chairpeople concerning IBC function;
- (3) the desirability of expediting reviews of proposals between RAC meetings and if so, how;
- (4) a proposal to incorporate the Physical Containment Recommendations for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules into the Guidelines as an Appendix;
- (5) the status of Recombinant DNA Advisory Committee (RAC) subcommittees and working groups; and
- (6) the current description of P4 physical containment.

Agricultural Concerns.

Dr. Nightingale asked Dr. Tolin to introduce the topic. Dr. Tolin began by questioning what RAC would envisage when it evaluates the introduction and dissemination of bioengineered plants into the environment. She noted the most of the important agricultural crops are not native to the U.S. and have been introduced.

Dr. Tolin said the U.S. Department of Agriculture (USDA), as well as university scientists and private individuals attempt to improve the germplasm of crops by collecting and maintaining specimens of the species. Collected seeds enter directly into the U.S. Plants and plant parts are subject to quarantine for periods of up to two years, until they are shown to be pathogen free. There are few restriction on importing germplasm. She then described breeding procedures, noting that limitations do exist on what can be crossed; some plants are self-pollinating, some are self-sterile, and some plants are only propagated vegetatively. She noted that traits such as yield are controlled by several genetic determinants; some traits, however, can be attributed to single genes. She said the situation in plants is more complicated than in mammals as many plants are polyploids, and thus, even if a trait can be attributed to a single inherited gene, multiple copies of that gene may be present.

Working groups and associations working with a particular crop species meet and determine procedures and guidelines for any particular species. Dr. Tolin said most breeding and selections are performed on land controlled by members of the group doing the research and testing. When the "elite" lines have been selected, these lines are then evaluated under varying conditions in field tests. Generally, these tests are conducted in several different areas around the country. State and Federal government and private industry are involved in this type of testing. State government laboratories usually work closely with agricultural experiment station and land-grant university scientists. These ventures, however, are primarily of an economic nature. State government laboratories test and rate seeds for trueness to type, germination potential, etc., and are often involved in seed distribution. They set standards for seed certification that are met by commercial seedsmen, often in accordance with local guidelines or laws. Vegetatively propagated plants follow the same scheme and often have more stringent requirements for certification (i.e., they must be virus free).

Dr. Martin asked Dr. Tolin if there were any instances of a plant originally thought to be beneficial, becoming a weed. Dr. Tolin offered the example of "Johnson grass" which was introduced into the U.S. because it is hardy and possesses a high stress tolerance. The grass, however, became a pest. It can be controlled, however, by herbicide application. Dr. Milewski noted that she had included the document "Spread of Organisms with Novel Genotypes: Thoughts from an Ecological Perspective" in the desk folders. This document discusses the introduction of Johnson grass and the establishment of other plants and organisms as pests in new ecosystems.

Dr. Martin asked if any problems had arisen from the current breeding procedures for crop plants. Dr. Tolin said that the U.S. corn crop had suffered heavy losses one year from blight caused by the fungus Helminthosporium maidis, race T. Because of its convenience the Texas male sterile trait was bred into most corn planted in the U.S., however, the trait renders the corn plant sensitive to a toxin produced by H. maidis. In that case, plant pathologists had warned against the exclusive use of the Texas male sterile trait. Dr. Tolin pointed out that plants and their pathogens coevolve, and it is difficult to predict what is going to happen.

Dr. Nightingale asked if the USDA had any guidelines on release of organisms into the environment. Dr. Tolin replied that some guidelines exist through the Plant Protection Quarantine Programs of APHIS, such as guidelines for the release of imported predators and pathogens for insect control. Recently, guidelines for introducing foreign organisms to control weeds have been formulated (Attachment I).

Dr. Scandalios mentioned the case of aflatoxin. Aflatoxin is a potent carcinogen produced by the fungus, Aspergillus flavus. The fungus infects the plants in the field and then when the grain is stored, grows. The plant breeders attack this problem in the traditional manner, i.e., breeding plants for resistance. However, a more fruitful method might be to study the interaction between the host and the fungus. He said "yellow rain" is another example of a fungal toxin which contaminates food crops.

Dr. Martin asked if RAC should become involved in this area: is the work new and unique; does systematic monitoring already exist; is there an agricultural working group? Dr. Scandalios replied that much new and unique work is becoming possible and suggested that some guidelines are necessary as none currently exist. He said genera now may be crossed using these technologies; this was not possible before the development of recombinant DNA techniques and protoplast fusion technology. Dr. Tolin pointed out that testing bioengineered plants will probably involve fewer individual plants than traditional breeding methods. She suggested it may be possible to draw containment guidelines on this basis; USDA did so for the field testing of bioengineered corn. Dr. Scandalios agreed; he said the distance of the test plants from other plants, the number of plants, etc, might be considered. Dr. Scandalios suggested that dissemination into the environment might be defined; he personally did not see field testing of 10 plants as release into the environment. Dr. Nightingale suggested that the Plant Working Group might be activated.

Dr. Nightingale asked Drs. Tolin and Scandalios if they might construct some general language to be presented to RAC. She suggested this language be published in the Federal Register and that experts in the field might be notified and asked to comment. Dr. Gartland noted that the language must be ready for publication at the end of February. He said the language of Section III-A-2 of the Guidelines would have to be modified.

Dr. Tolin said expression of a foreign gene by plants has just been reported, the Ti plasmid of Agrobacterium has been used to introduce the gene coding for kanamycin resistance into petunia plants. She suggested the working group

might wish to examine the question of expression of antibiotic resistance genes by plants. Dr. Scandalios said the question of spread of herbicide resistance genes is also important and should be considered. He suggested other plant specialists might be polled to determine if there are other concerns. Dr. Martin asked if a scenario existed for the spread of antibiotic resistance genes in plants and to animals. Dr. Tolin said she did not know of any mechanism of transfer. Dr. Nightingale asked if the statement that "genes in plant genomes are contained" is accurate, and if this can be used to redefine the idea of containment in plants. Dr. Scandalios suggested the working group might also evaluate questions concerning mitochondrial and chloroplast DNA. Dr. Gottesman questioned whether any introduced bioengineered species might become a pest and suggested this be considered. She asked Dr. Tolin if any introduced plant species have become problems. Dr. Tolin cited the example of Johnson grass. She said some introduced species have become pests following hybridization with native plants. Dr. Scandalios suggested the question of potential spread of the aflatoxin gene should be considered. He and Dr. Tolin suggested that an agricultural working group be called "Plants and Associated Organisms Working Group."

#### Status of RAC Subcommittees.

Dr. Nightingale said that the RAC charter currently authorizes three subcommittees and an indeterminate number of working groups. The working groups are constituted by the RAC chairman to serve a specific function and dissolved when no longer necessary. The subcommittees on the other hand are standing committees. She noted that two of the three subcommittees have not met for some time; furthermore, no issues will be placed before them in the foreseeable future. She wondered if some of the more pertinent working groups might not be substituted for these two subcommittees. She suggested the Working Group on Revision of the Guidelines and the Plants and Associated Organisms Working Group might replace the Host-Phage and the Host-Plasmid Subcommittees.

#### IBC Functioning.

Dr. Nightingale said ORDA had solicited responses from IBC chairpersons concerning:

- o problems with the revised guidelines;
- o what things are taking large amounts of time;
- o what things are taking inappropriate amounts of time;
- o in what areas do IBC chairpeople disagree with the RAC with regard to containment for a particular experiment;
- o in what ways are the Guidelines too stringent or too relaxed;
- o how frequently does the IBC meet;
- o does the IBC have other responsibilities at the institution.

Dr. Nightingale noted that to date, 10% of the IBCs polled have responded. This is not a good response rate, however most of the responses are supportive of the current Guidelines. She suggested if major problems had existed the response rate would probably have been higher as people generally do not write if they are satisfied.

Dr. Nightingale noted that some of the responses offered suggestions. These included:

- o that a condensed, one or two page guide to the guidelines be made available;
- o that ORDA communicate the basis for decisions;
- o that the language of Section III-B-4-a be clarified; and
- o that Appendix B be clarified.

Dr. Nightingale suggested that a condensed version of the Guidelines might be a good idea as principal investigators (PIs) new to the field must be educated in the Guidelines. Dr. Gottesman suggested this condensation might be either a table or statements indicating which portions of the Guidelines pertain to work with particular organisms. She thought both types of condensations might be reasonable.

#### Listing of Oncogenic Viruses in Appendix B.

Dr. Gartland called the group's attention to the listing of low and moderate risk viruses in Sections Appendix B-II-A and B-II-B. He noted that these viruses are not classified with a particular risk specification as are other agents in the Appendix. To further confuse matters, these viruses are listed between Class 4 and Class 5 agents. Dr. Martin said the National Cancer Institute (NCI) listing on which Appendices B-II-A and B-II-B are based is out-of-date. Dr. Gottesman noted that these viruses are not referred to in Section III of the Guidelines; Section III-B specifies containment for Class 2, 3, 4, or 5 agents while making no reference to low or moderate risk viruses. Dr. Nightingale suggested the working group recommend that the "Classification of Microorganisms Working Group" evaluate this issue. She asked if the composition of the Classification Working Group was adequate to evaluate the issue. The Working Group on Revision of the Guidelines agreed that several virologists should be appointed to the Classification Working Group. Dr. Tolin asked if Sections B-II-A and B-II-B in Appendix B might be deleted. Dr. Martin thought each virus in Section B-II-A and B-II-B should be individually evaluated. He noted that while some of the viruses might be safely handled at P1 physical containment, others such as HV ateles and HV saimiri should be handled under P3 or P4 containment conditions as they are known oncogenic viruses for higher primates.

Dr. Gottesman questioned whether the guidelines adequately addressed the question of virus hybrids created in vitro. Dr. Martin said no viable hybrids have been created by recombinant DNA technology. Dr. Gottesman asked if a hybrid virus containing a human oncogene could be created. Dr. Martin said the human oncogenes are very large, with introns, and that creation of a viable hybrid was not possible. Dr. Gottesman asked Dr. Martin if he thought the current guidelines were adequate except for intentional creation of hybrids. Dr. Martin replied that they were, helper virus would be required for propagation of the defective viruses created by introduction of the large cDNA coding for human oncogenes; this is covered by Section III-B-3.

Proposal to Incorporate the Physical Containment Recommendations for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules into the Guidelines.

Dr. Nightingale noted that the Large-Scale Review Working Group had forwarded to the Working Group on Revision of the Guidelines a proposal made at the October 26, 1982, meeting of the Large-Scale Review Working Group. That proposal would incorporate the Physical Containment Recommendations for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules into the NIH Guidelines.

Dr. Nightingale asked why the Large-Scale Recommendations had not been incorporated into the NIH Guidelines; she asked if there were any advantages to keeping the Recommendations a separate document. Dr. Milewski replied that the Recommendations, when formulated in 1980, applied primarily (and still do) to industry. Since industry is covered under Part VI of the Guidelines, Voluntary Compliance, including the Recommendations in the Guidelines was perceived as possibly inappropriate. Dr. Gottesman added that in 1980 it was thought leaving the Recommendations as recommendations would facilitate modification of the document.

Dr. Nightingale asked if any modifications to the Recommendations were required before they could be incorporated into the Guidelines. Dr. Gartland suggested that the language of the Recommendations was flexible and would not require substantive amendment. Drs. Milewski and Tolin said that the Recommendations required editorial updating and that this should be done before the Recommendations are incorporated into an Appendix of the Guidelines. Dr. Milewski said she would go through the Recommendations and send a memorandum to RAC for the next meeting concerning necessary editorial changes. The Working Group on Revision of the Guidelines agreed that the updated Recommendations might be added as an Appendix to the Guidelines.

Procedures for Reviewing Proposals Between RAC Meetings.

Dr. Nightingale noted that the period of time elapsing between RAC meetings is increasing. She wondered if some type of expedited review procedure, such as action by mail, phone, or by subcommittee or executive group, should be considered.

Dr. Gottesman suggested that if mail notification is used, any one RAC member might have veto power; the proposal would then be held for full RAC discussion and review. Dr. Nightingale agreed that one individual should have the authority to hold a proposal for RAC review and evaluation. Dr. Gottesman asked what

number of people would constitute an executive group. Dr. Scandalios said only 4 or 5 people can easily be handled by a conference call. He noted that these 4 or 5 people would have full RAC power so how individuals are selected to review proposals is very important. Dr. Gottesman said that a group of 6 might be reasonable; four experts, and two lay members. The RAC chairman should be part of every group. ORDA would rotate the members of the group. Should a RAC member express a desire to serve on the executive subcommittee reviewing a particular proposal, the member is automatically appointed to that subcommittee.

Dr. Gartland said that a similar procedure had been attempted in 1976 with the Host-Vector Subcommittee. This had not worked well. Dr. Martin said the questions currently facing the RAC are highly complex; he felt the current procedure should be followed, i.e., full RAC review, except for certain proposals in the agricultural area. The working group agreed.

#### Other Questions.

Dr. Tobin said that several investigators had questioned her on the Guidelines concerning the construction of "shuttle" vectors. These shuttle vectors are frequently constructed with DNA from 4 or 5 different species. Dr. Gottesman said that while protocols detailing the construction of these shuttle vectors may be complicated, the Guidelines specify that each piece of DNA must be evaluated. She saw no need to suggest modifying the Guidelines regarding the construction of shuttle vectors. She suggested that a statement on how to proceed might be included in any "condensation" of the Guidelines.

Dr. Martin questioned the specification in Appendix G-II-D-2-a on P4 containment. That Section specifies that "Experimental procedures involving organisms that require P4 level physical containment shall be conducted in (i) a Class III cabinet system or in (ii) Class I or Class II cabinets that are located in a specially designed area in which all personnel are required to wear one piece positive-pressure isolation suits." Dr. Martin said the specification requiring use of the Class III glove box is meant to protect the investigator against contamination by aerosol. He said the use of the Class III glove box, however, does not afford protection when infection by the organism being studied does not occur with aerosol exposure. This is the case with the E. coli K-12 host-vector systems. He suggested the language of G-II-D-2-a be amended to include a statement that "in those situations where an aerosol will not be generated or when illness is not caused by aerosol exposure, the research must be conducted in the P4 facility, but options for working outside the glove box may be available." Dr. Martin stressed that the P4 facility itself protects the environment, while the Class III glove box protects the investigator.

Dr. Gottesman asked if a contaminated investigator might carry the organism from the P4 facility into the community. Dr. Martin replied that use of P2 or P3 containment procedures in the P4 facility protects the community. He argued that automatic assignment of experiments to the glove box ties up NIH staff, since all manipulations are more difficult to perform in the glove box. Dr. Nightingale asked if Dr. Martin felt the local IBC ought to have the authority to set containment in the P4 facility on a case-by-case basis. Dr. Martin

replied that procedure is reasonable when E. coli K-12 and S. cerevisiae host-vector systems are used. He said all procedures involving animals should probably be assigned to the glove box. The working group agreed that language providing greater flexibility in use of the P4 facility should be introduced into Appendix G-II-D. This language should appear in the Federal Register for a period of comment.

Dr. Nightingale asked if the working group felt the periodicity of working group meetings was appropriate. The group agreed that twice yearly meetings between RAC meetings was an appropriate schedule for the Working Group on Revision of the Guidelines.

The meeting was adjourned at 12:10 p.m.

Respectively submitted,

March 11, 1983  
Date

Elizabeth Milewski  
Elizabeth Milewski, Ph.D.  
Executive Secretary

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

March 15, 1983  
Date

Elena Nightingale  
Elena Nightingale, Ph.D., M.D.  
Chairperson  
Working Group on Revision of the Guidelines  
of the Recombinant DNA Advisory Committee