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

RECOMBINANT DNA ADVISORY COMMITTEE
WORKING GROUP ON RELEASE IN THE ENVIRONMENT
MINUTES OF MEETING¹

MAY 31, 1984

The Working Group on Release into the Environment of the Recombinant DNA Advisory Committee (RAC) was convened at 10 a.m. on May 31, 1984, at the National Institutes of Health, 9000 Rockville Pike, Building 31, Room 5A16, Bethesda, Maryland 20205. The meeting was open to the public. Dr. Gerard McGarrity was Chair. The following people were present for all or part of the meeting:

Working Group Members:

Charles Arntzen	Robert Mitchell
Royston Clowes	Thomas Pirone
Nina Fedoroff	John Scandalios
John Fowle	Frances Sharples
Susan Gottesman	Sue Tolin
George Lacy	Anne Vidaver
Gerard McGarrity	William J. Gartland
Henry Miller	(Executive Secretary)

A working group roster is attached (Attachment I)

Other National Institutes of Health Staff:

Stanley Barban, NIAID
Elizabeth Milewski, NIAID

Other:

Fred Betz, Environmental Protection Agency
Anne Hollander, Environmental Protection Agency
Jane Rissler, Environmental Protection Agency
Mark Segal, Environmental Protection Agency

¹The Working Group is advisory to the RAC, and its recommendations should not be considered as final or accepted.

Dr. McGarrity called the Working Group on Release into the Environment to order at 10:10 a.m., May 31, 1984. He said the working group had three agenda items. These are: (1) to review the document "Proposed Guidelines for Submissions Under Appendix L" (Attachment II) which the working group had modified at their April 9, 1984, meeting; (2) to discuss plans for the risk assessment workshop to be sponsored by the National Institutes of Health (NIH) and the United States Department of Agriculture (USDA); and (3) to begin developing principles which could be applied to review of proposals involving field testing of genetically engineered microorganisms.

Dr. McGarrity called the attention of the working group to a document (Attachment III) which had been submitted to the working group by the Environmental Protection Agency (EPA) representative, Dr. John Fowle. Dr. Fowle of the EPA Office of Research and Development (ORD) said the EPA document is to provide points for industry to consider in preparing premanufacture notices for genetically engineered organisms which may be reviewed under the Toxic Substances Control Act (TSCA). The objective of the document is to develop general guidelines and provide general guidance for evaluating genetically engineered organisms proposed for release into the environment and is intended to be flexible and evolve over time. Dr. Fowle said EPA is forwarding the document to the Working Group on Release into Environment to solicit comments from the working group and to provide information which might be useful to the group. Eventually, EPA plans to solicit wide public comment on the document by publishing it as technical background to the planned Federal Register notice, currently scheduled for publication this fall.

Dr. Scandalios asked who had developed the EPA document. Dr. Hollander of the EPA Office of Toxic Substances (OTS) said OTS and ORD had developed it. The document is in a very early stage of development, but EPA feels it is appropriate to share its thoughts with the working group. She emphasized that the document is restricted and not for general distribution.

REVIEW OF THE "PROPOSED GUIDELINES FOR SUBMISSION UNDER APPENDIX L"

Dr. McGarrity asked Dr. Tolin to review the history of those documents dealing with field testing of plants modified through recombinant DNA techniques. Dr. Tolin said the first proposal to field test a genetically modified plant was submitted to the Recombinant DNA Advisory Committee (RAC) by Dr. Ronald Davis of Stanford University. Dr. Davis requested permission to field test corn plants (Zea mays) which had been transformed by corn DNA or modified corn sequences. The RAC reviewed this proposal at its June 5-6, 1980, meeting and recommended approval. However, the Director, NIH, in the Federal Register of July 29, 1980, announced that he was deferring action on this recommendation pending receipt of additional information on technical aspects of the experiments. Dr. Tolin said the issue was referred to the Department of Agriculture, and as the USDA representative to the RAC, she wrote the following to the NIH on August 7, 1980:

"Corn (Zea mays) is not known to exchange genetic information with any other species. Furthermore, pollination of corn can be carefully controlled since male and female flowers are borne separately on the same plant. Tassels can be made sterile or pollen from fertile tassels can be contained. Silks on ear shoots can be covered so that no or only desired pollen fertilizes the ovules. The hybrid seed corn industry is based on these biological facts."

Dr. Tolin said she wrote that USDA favored approving this request, but that certain specified practices should be followed.

In June and July of 1981, Dr. Davis and his colleague, Dr. Virginia Walbot, provided additional technical information on the proposed experiments including detailed information on hosts and vectors, DNA transfection methods, the location of test fields and containment procedures. Following receipt and review of this information the USDA Recombinant DNA Committee recommended that Dr. Davis and his colleagues be permitted to proceed under specified conditions.

The NIH granted Dr. Davis permission to proceed with this field test by a notice in the Federal Register on August 7, 1981, on the basis that it presented no significant risk to health or the environment. Language indicating this permission was added to Appendix D of the Guidelines.

Dr. Tolin said RAC then received in June 1982 a request from Dr. John Sanford of Cornell University for permission to field test tomato and tobacco plants transformed with bacterial (E. coli K-12) and yeast (Saccharomyces cerevisiae) DNA using pollen as a vector. The RAC reviewed this request at its meeting on October 25, 1982. During the discussion, it was stated that the probability the experiments would be successful is very low, but should the procedure be successful, no hazard was foreseen, and RAC recommended approval.

Final action on the recommendation was deferred by the NIH pending a review of the proposal by the USDA Recombinant DNA Committee. The USDA Recombinant DNA Committee met on February 23, 1983, and discussed Dr. Sanford's proposal at that meeting. The USDA Committee unanimously approved Dr. Sanford's proposal and could foresee no potential hazard to humans or the environment as a result of performing these experiments in the fields at Geneva, New York.

The NIH accepted the recommendations of the RAC and the USDA Recombinant DNA Committee and officially added, by a notice in the Federal Register on April 15, 1983, language to the Guidelines (Appendix D) granting permission to Dr. Sanford to field test tomato and tobacco plants transformed with bacterial (E. coli K-12) and yeast DNA on the basis that the proposed experiments presented no significant risk to health or the environment.

Dr. Tolin said as RAC had predicted, greenhouse and growth chamber experiments were not successful, and Dr. Sanford has no plans to proceed with field testing.

Dr. Tolin said RAC subsequently requested that a document be prepared which would specify the types of generic information RAC would review in evaluating

experiments involving field testing of genetically modified plants. The Plant Working Group and USDA representatives prepared a document which was published in the March 4, 1983, Federal Register. The document specified that provided the experiments met the criteria detailed in the document, the IBC could review the proposal; ORDA would be notified of approvals. The RAC considered the Plant Working Group proposal at its April 11, 1983, meeting and discussed it extensively. The RAC made several modifications in the specific criteria as the result of scientific considerations. The RAC also modified the procedural aspects of the proposal. It recommended: (1) the language be incorporated into the Guidelines as a new Appendix L, and (2) proposals must be reviewed and approved by the Plant Working Group and by the IBC before initiation of experiments.

The NIH accepted this recommendation and Appendix L appeared in the June 1, 1983, Federal Register (48 FR 24549).

Dr. Tolin noted that any proposal involving plants which does not meet the criteria specified in Appendix L must be reviewed and recommended by the full RAC on a case-by-case basis.

Dr. Gartland said the guidance document (Attachment II) the Working Group on Release into Environment would consider at the May 31, 1984 meeting was a support document to Appendix L. He said this guidance document entitled "Proposed Guidelines for Submission Under Appendix L" was originally developed by the Plant Working Group to supplement the information found in Appendix L. This guidance document was sent to RAC for consideration at the February 6, 1984, RAC meeting. During the RAC discussion, several comments and recommendations were made concerning the document. He said the Working Group on Release into Environment at its April 9, 1984, meeting subsequently modified this document. He asked the Working Group on Release into Environment to consider whether the comments and recommendations made at the February 6 RAC meeting had been incorporated into the document.

Dr. Gartland said the document "Proposed Guidelines for Submissions Under Appendix L" would be a source of information and would probably be published in the Recombinant DNA Technical Bulletin and sent to IBC chairpeople. It would not be incorporated into the Guidelines.

Dr. Gottesman felt that the comments and recommendations made by RAC at the February 6 meeting had been incorporated into the document. Other working group members agreed. Dr. Gottesman suggested, however, that the third paragraph of the document be modified. That paragraph reads as follows:

"These annotated items were presented for consideration by prospective proposal submitters to facilitate the process of approval. The Working Group has found that the proposals so far submitted for their consideration have omitted information that is considered minimal and essential for their approval. Basically, the group would like to see detailed objectives, materials and methods, including methodology for monitoring the experiments, and expected results. At a minimum summary data should be submitted to

support the proposal. A check list of detailed requirements should include, but is not limited to...."

Dr. Gottesman moved that the second sentence and the word "basically" of the third sentence be deleted. Dr. Arntzen seconded the motion. Dr. Sharples questioned whether the first sentence of the paragraph was relevant. Dr. Gottesman agreed that the first sentence added little to the paragraph, and thought it preferable to modify all of the third paragraph. She suggested that language notifying investigators that plant experiments not covered by Appendix L would be reviewed by RAC on a case-by-case basis be included in this paragraph.

Dr. Fedoroff suggested the first and second sentence of that paragraph be deleted. She thought the third sentence should read:

"The working group should receive a description of objectives, materials, and methods including methodology for monitoring the experiments and expected results."

Dr. Miller said "objectives" should be "stated" while materials and methods are "described;" he suggested Dr. Fedoroff's proposed language be amended to include this distinction. Dr. Gottesman amended her motion to include these suggested modifications. Dr. Fedoroff suggested the fourth sentence of the paragraph be modified to explicitly request information on preliminary data and expected results. Dr. Scandalios agreed. Dr. Arntzen suggested the following sentence be substituted for the fourth sentence:

"A summary of relevant preliminary results should accompany the results."

Dr. Gottesman accepted this recommendation. She suggested the second paragraph and the subtitle which follows the second paragraph be deleted. That language reads:

"A RAC Working Group has now prepared draft submission guidelines for individuals preparing proposals under Appendix L of the Guidelines. This proposed guidance is as follows:

"Items for Consideration to be Included in Proposal Submissions Under Appendix L."

Dr. Gottesman thought the proposed language should provide a "core" of information; it should thus include information on experiments involving field testing of plants not covered under Appendix L. Dr. Sharples did not think the proposed first paragraph of the guidance document should refer to experiments not covered by Appendix L as the body of the guidance document does not request information necessary for evaluating such experiments. For example, the guidance document does not require information on the ecology of wild plants; information of this type should be evaluated in reviewing experiments involving plants not covered by Appendix L.

Dr. Clowes asked Dr. Tolin to describe the types of experiments covered by Appendix L. Dr. Tolin said Appendix L applies to plant species which are cultivated crops of a genus that has no species known to be a noxious weed. These requirements are described in Appendix L-II-A.

Dr. Clowes asked if RAC will in the near future receive proposals involving field testing of plants not covered by Appendix L-II-A. Dr. Fedoroff suggested that investigators may wish to field test modified Arabidopsis. Arabidopsis is a known weed, albeit a highly studied plant.

Dr. Clowes asked if all crop plants are covered by Appendix L. Dr. Tolin replied that most crop plants will be covered, but potatoes and rice are not covered by Appendix L.

Drs. Clowes and Fedoroff asked why Appendix L applies only to cultivated crop plants of a genus that has no species known to be a noxious weed. Dr. Tolin said this specification was developed following a USDA recommendation. What is known about how plants become weeds indicates that this process generally follows taxonomic classifications.

Dr. Pirone felt the proposed guidance document should be specific and only refer to cultivated crop plants of a genus that has no species known to be a noxious weed; documents dealing with experiments involving other plants should be constructed at a later date. Drs. McGarrity and Tolin suggested the working group would be most successful if it concentrated on one task at a time; they urged the working group to concentrate its efforts on developing guidance for proposals submitted under Appendix L. The working group agreed.

Dr. Gottesman reread her modified motion concerning the title and the introduction to the guidance document. The motion read as follows:

"Proposed Guidelines for Submission Under Appendix L.

"Appendix L of the Guidelines specifies conditions under which certain plants may be approved for 'release into the environment' including field tests. Experiments in this category cannot be initiated without submission of relevant information on the proposed experiments to NIH, review by the RAC Plant Working Group, and specific approval by NIH.

"The proposal should include a statement of objectives and a description of materials and methods including methodology for monitoring the experiments and expected results. A summary of relevant preliminary results should accompany the proposal. A check list of detailed requirements should include but is not limited to...."

Dr. Scandalios seconded the modified motion. By a vote of twelve in favor, none opposed, and no abstentions, the working group accepted the motion.

Dr. McGarrity suggested the working group proceed through the document evaluating each item. He called the group's attention to item A, "Description

of Plant Materials," and began by asking whether item A-1 was adequate. That item reads as follows:

"'Tomato plants will be inoculated' is insufficient. Give common and scientific names of plants and cultivars if appropriate."

Dr. Scandalios said simply requiring the identity of "cultivars" would not completely identify the genetic origin. Dr. Tolin said genetic origin can be described by the terms "line," "introduction," "seeds," "hybrid," etc. Dr. Vidaver said item A-1 should require a description sufficient to permit the plant to be identified. Dr. Segal asked if the phrase "complete and common nomenclature" would be adequate. Dr. Tolin replied that more than complete nomenclature would be required to identify the genetic origin. Dr. Pirone suggested the phrase "cultivars and genetic lines" be used. Dr. Clowes suggested the words "identify by" be substituted for the word "give."

Dr. Lacy asked if the second sentence of item A-1 is necessary. Drs. Vidaver and Tolin replied that this sentence was included for historical reasons; the Plant Working Group had often found descriptions in proposals submitted for review rudimentary. Dr. Fedoroff felt this type of directive was unnecessary and could be deleted without affecting the informational content of the guidance document. Drs. Tolin and Vidaver agreed to delete the second sentence.

Dr. McGarrity then called the working group's attention to item A-2 which reads as follows:

"If appropriate, give data or information on the relative homogeneity of the plant cultivar, and specific genetic markers the cultivar is known to possess."

Dr. Arntzen felt item A-2 should request relevant data. Dr. Fedoroff felt item A-2 should be incorporated into item A-1. Dr. Scandalios said item A-2 requests different information than item A-1; for this reason, it may be preferable to have two separate statements. Dr. Fedoroff suggested A-1 and A-2 could be combined as follows:

"Give common and scientific names of plants. Identify the specific cultivars or genetic lines to be used. Include information on the relative homogeneity of the plant cultivars or lines and specific genetic markers they are known to possess."

The working group agreed to accept Dr. Fedoroff's proposed language.

Dr. McGarrity then called the working group's attention to items B-1 and B-2 of Section B, "Vectors and Method of Introduction." Items B-1 and B-2 read as follows:

"1. Describe the cloned DNA segment and its expression in the new host.

- "2. Give the method(s) by which the proposed DNA vector will be or has been constructed. Diagrams are very helpful and may be necessary for adequate understanding of the construct. Explain the advantages (and disadvantage(s), if appropriate) of your vectors, if other candidate vectors could be considered."

Dr. Lacy suggested the second sentence of item B-2 be deleted. He thought a statement such as "[Use diagrams for clarity]" was adequate. Dr. Miller thought such a statement would imply the working group was only interested in diagrams.

Dr. Vidaver feared Dr. Lacy's proposed statement was vague and the working group might receive diagrams which are not pertinent. Dr. Arntzen suggested the first and second sentences of item B-2 are clear and should not be modified. Dr. Vidaver agreed. Dr. McGarrity asked if the third sentence of item B-2 was acceptable. Dr. Gartland suggested the word "describe" be substituted for the word "explain" in the third sentence. The working group agreed that the language of items B-1 and B-2 was adequate, but that the word "describe" would be substituted for the word "explain."

The working group then considered the language of item B-3 which read as follows:

"If microorganisms are used to introduce vectors or are vectors themselves, indicate how they compare with wild-type strains. If disabled pathogens are used to transmit the vector, indicate measures that will most likely prevent these microorganisms from regaining or acquiring pathogenic potential. If the vector is likely to survive independently of the hosts, refer to this possibility, and provide any available data to assess the probability of transfer to likely organisms."

Dr. Fedoroff asked if the definition of a vector should be included in this item. Dr. Gottesman felt the language of item B-3 was adequate. She pointed out that vectors for modifying plants would include the bacteria, Agrobacterium tumefaciens, and the virus, Cauliflower Mosaic Virus, and an inclusive definition of a vector could become very detailed.

Dr. Clowes questioned the use of the word "measures." This usage suggested to him "physical measures to prevent spread." He thought intrinsic characteristics of the organisms should also be considered. He suggested the word "characteristics" be substituted for "measures." Dr. Sharples suggested the word "factor" be used. Dr. Clowes agreed.

Dr. Sharples questioned the use of the word "such" in the last sentence of item B-3. Dr. Arntzen suggested the word "likely" be substituted for "such." Dr. Lacy suggested the word "host" in the third sentence of item B-3 be qualified by the word "desired." The working group agreed to these proposed modifications.

The working group then evaluated item B-4 of "Vectors and Methods of Introduction" which reads as follows:

"If microorganisms are used to introduce vectors, the assessment of subsequent absence of the microorganisms should be specified. Indicate the means of strain identification and retrieval."

Dr. Segal questioned whether this language addressed the concept of the effect culture homogeneity would have on efforts to assess the subsequent absence of the microorganism. Dr. Lacy said the language of item C-1 of the guidance document includes this concept.

Dr. Clowes suggested the word "persistence" be substituted for the word "absence" in the first sentence of item B-4. Dr. Sharples asked if item B-4 stated that the plants to be field tested should be free of microorganisms if these are used to introduced the recombinant DNA. Dr. Tolin asked if the working group felt plants containing such microorganisms could be placed in the environment. The working group agreed that this document should specify conditions for field testing of plants, it would not deal with the introduction of associated microorganisms to the environment, thus, the sentence as originally written is acceptable. Dr. Gottesman suggested the first sentence might read:

"If microorganisms are used to introduce vectors, describe how the absence of the microorganisms was assessed."

Dr. Pirone elaborated on Dr. Gottesman's proposed language and suggested the first sentence of item B-4 might read:

"If microorganisms are used to introduce vectors, the absence of these microorganisms in plants to be released in the field should be documented."

He suggested that the second sentence of item B-4 be deleted.

The working group agreed to these modifications.

Dr. McGarrity called the attention of the working group to item C-1 of Section C, "Characteristics and Monitoring of Plants." Item C-1 reads as follows:

"Provide data from greenhouse and/or growth chamber studies under simulated field conditions to support prospective field studies. Data should include morphological data for at least two generations of plants.

"Specify plant monitoring procedures; frequency; types of data to be obtained, including leaf, seed, fruit or root characteristics."

Dr. Fedoroff pointed out that growth chamber conditions do not simulate field conditions. She asked whether conditions could be specified which would actually simulate field conditions and questioned the use of the phrase "under simulated field conditions." Dr. Scandalios suggested the phrase "simulated field conditions" be deleted from the first paragraph. Dr. Vidaver pointed out that if this phrase were deleted data might be submitted which are clearly not applicable to field testing. Dr. Gottesman agreed with Dr. Vidaver but thought it would be difficult to specify conditions which would create "simulated

field conditions." The working group agreed to delete the reference to "simulated field conditions."

Dr. Fedoroff suggested the first two words of the second sentence of item C-1 be deleted. Dr. Segal asked if a request for morphological data would result in adequate information being provided for review. Dr. Lacy said he would like to know if the construct is stable, and morphological data would not provide this information. Dr. Fedoroff thought the document should request information on the fate of the recombinant DNA. Dr. Arntzen said the document should specify a requirement for data dealing with any evidence of changes in traits. Dr. Tolin felt the document should not require complete genetic information as this would result in large amounts of data being submitted for review. Dr. Clowes thought the working group would be primarily interested in reviewing data on the DNA insert and the vector. Dr. Fedoroff said that in certain cases the working group might wish to review all available information including the complete genetic background of the plant. She thought the investigators should provide any available information particularly any existing data on the molecular characterization of the plants.

Dr. Arntzen suggested the second sentence of item C-1 might read:

"Supply existing molecular, physiological, or morphological data as applicable to the trait(s) under consideration."

Dr. Tolin asked if Dr. Arntzen's proposed language would include a statement requiring data for at least two generations of plants. Dr. Arntzen questioned whether a statement requiring data over two generations should be specified in item C-1. He pointed out that in some cases it would not be feasible to generate such data. He said some crop plants such as pine trees have long generation cycles; one might begin the experiment as a graduate student and be retired before the requisite data had been generated. Dr. Tolin suggested the guidance document might require data covering two cell cycles rather than two generations. Dr. Lacy felt the specification for data covering at least two generations of plants should remain in the document but be qualified by the phrase "when applicable." Drs. Arntzen and Pirone thought the phrase "if feasible" preferable to the phrase "when applicable." After further discussion the group agreed to use the phrase "if feasible."

Dr. Hollander asked if the language of item C-1 would specify that the investigators examine and report any unexpected results. Dr. Tolin said language could be added to item C-1 requesting that "strange observations be reported." Dr. Pirone suggested the working group could ask the investigators to "show how care has been taken to show that no undesirable traits will develop."

Dr. Gottesman suggested these concerns could be addressed by rewriting the second sentence of item C-1 as follows:

"Include morphological data for at least two generations of plants, if feasible. Supply any molecular or physiological data, especially as applicable to the trait(s) under consideration."

She asked whether this language suggests the plants should be observed for any abnormalities or unexpected results. The group agreed it did. Dr. Arntzen said the word "abnormality" is not used in the sense of pathology; rather the investigator would be looking for abnormalities in physiology. Dr. Scandalios felt the word "abnormalities" should not be stressed. He said in plants what might appear to be abnormal in some situations may actually be normal. He gave as an example the situation involving anther ears in corn grown in greenhouses. Corn rarely has anther ears in the field, however, corn commonly develops anther ears when grown in the greenhouse. To investigators unfamiliar with the growth characteristics of the plant, this might appear to be "abnormal." Dr. Tolin said this is an example of how the greenhouse response may differ from the response in the field. She said many plants cannot be tested in the greenhouse for this reason.

Dr. Arntzen suggested the second paragraph of item C-1 should read as follows:

"Specify plant monitoring procedures, frequency, types of data obtained."

The working group agreed to this modification.

Dr. McGarrity then called the attention of the working group to item C-2. Item C-2 reads:

"Provide data for field plot design on the following:

"a. total area;

"b. location: where, how many;

"c. plot design: e.g., replication, row spacing, planting, border rows, etc.;

"d. name cultivar(s), if appropriate;

"e. specify plant monitoring procedures: frequency; types of data to be obtained, including leaf, seed, fruit, or root characteristics; abnormalities such as diseases; insect population monitoring; collection of meteorological data etc.; types of data to be sought such as yield, resistance to stress, lodging, etc.;

"f. specify monitoring of the vector and/or introduced DNA; and

"g. specify access and security measures."

Dr. Gartland asked if item C-2-b referred to geographical location. The working group agreed it did, and suggested the word "geographical" be added to item C-2-b.

Dr. Pirone felt item C-2 should ask if any commercial crops are being grown near the testing area. Dr. Lacy said he would like to know the character and type of

the surrounding plant culture or plant habitat. Dr. Tolin said in reviewing applications RAC and the Plant Working Group have questioned investigators as to the proximity of the testing fields to commercial production areas. In requests involving modified plants, RAC has accepted fields removed from commercial production areas as testing plots.

Dr. Tolin felt the working group would not be interested in evaluating the potential impact of plants covered by Appendix L on all surrounding plants, including weeds. Dr. Lacy felt in certain cases one should consider whether plants other than commercial species are near to the test fields. He offered as an example the possible interaction of test orchards which might be located near forests with plants in the forests. Dr. Arntzen felt his primary concern would be that test fields be removed from areas of commercial agricultural production. Dr. Pirone felt the working group would not be concerned about the proximity of local gardens.

Dr. Tolin asked if Dr. Alexander's comment at the February 6 RAC meeting concerning monitoring of soils was pertinent to Section C. Dr. Vidaver said in her reply to Dr. Alexander's comments she had suggested that the following language be added to item C-2:

"...ecological factors that would be likely to affect the potential success, such as soil etc., should be monitored."

Dr. Fedoroff questioned whether the working group should be concerned with the success or failure of the field test. Dr. Gottesman replied that if the plants grow well in the testing environment, one might then be concerned that they would survive well in other environments. Should they grow well, one would evaluate the potential for spread of the engineered plant into the environment.

Dr. Gottesman asked if ecological aspects or conditions might be important in controlling potential environmental spread; for example, would conditions such as humidity favor persistence. Dr. Lacy felt the language should emphasize factors which might affect "containment" of the plant.

Dr. Fedoroff said Appendix L applies to cultivated crops dependent on humans for propagation. She said the most important consideration in evaluating experiments involving field testing of plants is how the engineered plant differs from the non-engineered parent. She felt there would be little difference between engineered and non-engineered crop plants dependent upon man for propagation. Genetic engineering will not convert tobacco into kudzu.

Dr. Arntzen suggested the following compromise language should be included in item C-2:

"If applicable for the trait under study, ecological information on factors such as water, soil, etc., should be provided."

Dr. Tolin pointed out that Appendix L-II-D succinctly states the appropriate conditions for field testing plants under Appendix L. Dr. Gottesman agreed. Dr. McGarrity suggested that the language of Appendix L-II-D be restated in the guidance document.

Dr. Pirone questioned whether a specification requiring a description of plot design should be included in item C-2-c. He thought a study section would review this information as part of the scientific review and, thus, the working group would not need to review such information. Dr. Scandalios said this assumption does not always hold true. He pointed out that the study section reviewing the proposal involving field testing of modified corn plants did not consider the conditions under which the corn would be grown, although the experimental design was critical to the outcome of the test. If the field design was not reviewed when it was pertinent to the outcome, he doubted it would be reviewed in other cases. He felt the RAC guidance document should require information on the experimental design. Dr. Tolin felt such information should be requested. She said proposals had to be submitted to RAC for a second review at the June 1, 1984, meeting because the original proposals did not contain an adequate design for generating and assessing field plot data.

Dr. Gottesman agreed and suggested that item C-2 should consist of the language of Appendix L-II-D and the language included in items C-2-a through C-2-f. The working group agreed.

Dr. Tolin thought the word "planting" should be deleted from item C-2-c. Dr. Vidaver suggested the "etc." be deleted from item C-2-c, and that an "e.g." be included.

Dr. Arntzen suggested that item C-2-d was redundant and should be deleted. The working group agreed this item was redundant. Dr. Arntzen suggested the words "nature of" be added to modify the phrase "border rows" in item C-2-c. He also suggested the term "abnormalities such as" be deleted from item C-2-e. He reiterated his opinion that the guidance document should not emphasize the concept of "abnormality." Dr. Scandalios agreed.

Dr. Arntzen suggested that the requirement for "types of data to be sought, such as yield, resistance to stress, lodging, etc." should be deleted from item C-2-c as this concern is addressed elsewhere.

Dr. Rissler pointed out that the document being generated by the working group only requires data on field testing in "average" conditions. No data will be generated on plants grown in "extreme" ecological conditions, and it will not be known whether the engineered plants might survive and grow under conditions in which the nonengineered parent plant might not grow. Dr. Gottesman said Appendix L only applies to testing in a specified field plot. Data generated by other testing procedures would be required for review and approval for testing and use in other locations.

Dr. Fedoroff called the working group's attention to the fact that while the concept of controls is implicit in the guidance document, this consideration is not explicitly stated. She felt the document should explicitly state a

requirement for use of controls. Drs. Tolin, Arntzen, and Pirone agreed. Dr. Arntzen said a statement concerning use of controls would logically be inserted in item C. Dr. Pirone suggested item C-1 might include a statement to the effect that "data should include information on engineered and control plants." Dr. Scandalios felt the title of Section C should be modified to read:

"Characteristics and Monitoring of Genetically Engineered and Control Plants."

The working group accepted Dr. Scandalios' suggestion.

Dr. Arntzen questioned whether the working group should specifically request that monitoring techniques be described. Dr. Fedoroff felt inclusion of a specific statement was unnecessary; she thought item C-2 was specifically saying "tell us how you monitor." She felt the question of whether the proposed monitoring was adequate should be addressed on a case-by-case basis.

Dr. Scandalios felt the proposed modification and the genetic stability of the inserted DNA should be evaluated. Dr. Tolin said Appendix L-II-C specifies the types of modifications which may be introduced into the test plants under Appendix L. Dr. Lacy felt "changes" could involve deletion as well as insertion of genetic materials. He suggested the term "altered DNA" was more encompassing and should be introduced into item C-2-f. The working group agreed.

Following this discussion the Working Group on Release into Environment agreed the guidance document would read as follows:

"Points to Consider for Submission Under Appendix L.

"Appendix L of the Guidelines specifies conditions under which certain plants may be approved for 'release into the environment' including field tests. Experiments in this category cannot be initiated without submission of relevant information on the proposed experiments to NIH, review by the RAC Plant Working Group, and specific approval by NIH.

"The proposal should include a statement of objectives and a description of materials and methods, including methodology for monitoring the experiments, and expected results. A summary of relevant preliminary results should accompany the proposal. Information to be submitted should include but not be limited to:

"A. Description of Plant Materials.

Give common and scientific names of plants. Identify the specific cultivars or genetic lines to be used. Include information on the relative homogeneity of the plant cultivars or lines and specific genetic markers they are known to possess.

"B. Vectors and Method of Introduction.

- "1. Describe the cloned DNA segment and its expression in the new host.
- "2. Describe the method(s) by which the proposed DNA vector will be or has been constructed. Diagrams are very helpful and may be necessary for adequate understanding of the construct. Explain the advantages (and disadvantage(s), if appropriate) of your vectors, if other candidate vectors could be considered.
- "3. If microorganisms are used to introduce vectors or are vectors themselves, indicate how they compare with wild-type strains. If disabled pathogens are used to transmit the vector, indicate factors that will most likely prevent these microorganisms from regaining or acquiring pathogenic potential. If the vector is likely to survive independently of the desired host(s), refer to this possibility and provide any available data to assess the probability of transfer to other organisms.
- "4. If microorganisms are used to introduce vectors, the absence of these microorganisms in the plants to be released in the field should be documented.

"C. Characteristics and Monitoring of Genetically Engineered and Control Plants.

- "1. Provide data from greenhouse and/or growth chamber studies to support prospective field studies. Include morphological data for at least two generations of plants as appropriate. Supply any molecular or physiological data, especially as applicable to the trait(s) under consideration.

"Specify plant monitoring procedures, frequency, and types of data obtained.

- "2. Field plots should meet the criteria specified in Appendix L-II-D:

"Appendix L-II-D. Plants are grown in controlled access fields under specified conditions appropriate for the plant under study and the geographical location. Such conditions should include provisions for using good cultural and pest control practices, for physical isolation from plants of the same species outside of the experimental plot in accordance with pollination characteristics of the species, and for further preventing plants containing recombinant DNA from becoming established in the environment. Review by the IBC should include an appraisal by scientists knowledgeable of the crop, its production practices, and the local geographical conditions. Procedures for assessing alterations in and the spread of organisms containing recombinant DNA must be

developed. The results of the outlined tests must be submitted for review by the IBC. Copies must also be submitted to the Plant Working Group of the RAC.

"Supporting data should include the following:

- "a. total area;
- "b. geographical location(s): where, how many locations;
- "c. plot design: for example, replication, row spacing, nature of border rows;
- "d. specify plant monitoring procedures: frequency; types of data to be obtained, including leaf, seed, fruit, or root characteristics; disease, insect and other animal population monitoring as appropriate.
- "e. specify techniques for monitoring the vector and/or altered DNA; and
- "f. specify access and security measures."

RISK ASSESSMENT WORKSHOP

Dr. McGarrity asked Dr. Tolin for an update on the planned risk assessment workshop. Dr. Tolin said the workshop planned jointly by the NIH and USDA was to review and synthesize available scientific information. She said the NIH-USDA workshop should provide information to RAC in its deliberations and should also benefit RAC working groups such as the Working Group on Release into Environment. Dr. Tolin thought the workshop would focus primarily on plants and associated microorganisms and would most probably be similar in format to the workshop sponsored by the National Institute of Allergy and Infectious Diseases (NIAID) at Pasadena, California.

On April 11-12, 1980, NIAID sponsored in Pasadena, California, A "Workshop on Recombinant DNA Risk Assessment." The workshop was designed to define the scientific issues and assess the potential risks of: (1) possible direct adverse effects of hormone-producing strains of E. coli K-12, and (2) the possible occurrence of autoantibodies or autoreactive cells due to the production of eukarotic polypeptides (including hormones) by E. coli K-12 should such strains for unexpected reasons colonize higher organisms. In order to address these topics, the meeting brought together scientists from the fields of immunology, endocrinology, physiology, microbiology, infectious diseases, and other appropriate disciplines. The information synthesized by the workshop and workshop recommendations to NIAID were used to implement the NIH program to assess the risks of recombinant DNA.

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Dr. McGarrity called the working group's attention to a letter (Attachment IV) from Representative Albert Gore (D-Tenn) to Dr. Robert P. Williams, the President of the American Society for Microbiology (ASM). In his letter, Representative Gore requested that ASM convene a national symposium to examine scientific issues involved in releasing genetically modified organisms into the environment. Mr. Gore wrote:

"Generally, the purpose of the conference would be to help identify the scientific issues of relevance and to stimulate debate in the appropriate scientific disciplines. More specifically, the conference should:

- "1) attempt to define the range of impacts of deliberately released organisms;
- "2) differentiate, if possible, between effects of engineered organisms returned to their own locations and translocated organisms, whether engineered or not; and
- "3) discuss the usefulness of existing methodology for estimating the above effects in terms of precision and accuracy.

"Ideally, the conference will result in the publication of a collection of papers by and for scientists and a summary for a lay audience. The final document would also contain an overview of methods of producing genetically engineered organisms, in terms of interactions with ecosystems, since one of the more perplexing problems is setting limits on the problem area."

Dr. Gartland said he had received a copy of Representative Gore's letter on May 30, 1984, and had no chance to investigate the question before the working group meeting. He asked if ASM is likely to sponsor a conference dealing with deliberate release of plants.

Dr. Tolin said ASM currently is heavily oriented towards medical microbiology. Dr. Clowes said ASM supports conferences through sales of volumes of the proceedings. He wondered if there is currently enough information available to produce a volume. Dr. Gottesman thought most available data are in the form of case studies; those studies could be included in a volume whether they are relevant or not to pertinent questions. She felt ASM should be contacted to determine whether ASM is planning a conference and whether the subject matter of the proposed ASM conference would overlap with the subject matter of the proposed NIH-USDA workshop.

Dr. Tolin asked the working group to offer suggestions about the type of information which should be discussed or the questions they would like to see addressed at the proposed NIH-USDA workshop.

Dr. Sharples said slight genetic changes can alter significantly the impact of an organism on the environment. She pointed to the single gene changes which occur in pesticide and antibiotic resistance. She felt these types of questions should be considered.

Dr. Fedoroff said the modification of crop plants through use of the recombinant DNA technique is comparable to introducing new lines developed through standard agricultural breeding techniques. Dr. Gottesman suggested breeders should at the workshop present information about the effect of introducing new lines developed through standard breeding techniques.

Dr. Scandalios said he would like to see a presentation on the effect of the introduction of soybeans to the U.S. He felt this discussion would offer insight on the ecological effects of the introduction of cultivated crop plants.

Dr. Scandalios thought the question of what effect a one gene modification might have on higher plants could be addressed by examining the history of the use of the male sterile characteristic in corn. Dr. Fedoroff said a blight pathogen became epidemic on corn possessing the male sterile trait. She thought this type of event was one of the "worst case scenarios" for higher plants, however, this development was due to the agronomic practice of monoculture. Dr. Lacy said agricultural practices have a great influence on agricultural ecology. He said monoculture, which is economically effective, affects the types of pathogens which develop and the ecology as a whole. Dr. Pirone said monoculture of male sterile corn resulted in selection from the natural pathogen population of a variant highly virulent for corn with that characteristic. He said this type of occurrence is a fact of life in agriculture and keeps the plant breeders occupied. He did not think plants modified by recombinant DNA technology would present problems different from those already present in agriculture. He said he would ask the workshop to address the question, "How would genetically engineered plants differ from other plants?"

Dr. Pirone felt the characteristics of plants developed by standard breeding techniques were far less predictable than those developed using recombinant DNA techniques.

Dr. Sharples said the recombinant DNA technique could introduce characteristics into plants which might not otherwise acquire them. She wondered if events such as transfer of the characteristic to other organisms, perhaps by the transfer of the recombinant vector, would be a concern.

Dr. Scandalios suggested workshop speakers might present information on the characteristics and behavior of plants modified using standard breeding techniques; individuals having ecological concerns should also participate in order to fully examine the issues. Dr. Gottesman agreed and said the workshop could contrast the questions posed by Dr. Sharples with the experience of individuals associated with traditional plant breeding. She thought the proposed workshop should be structured as the Pasadena workshop was structured, i.e., the limits of concern should be defined. She did not think papers and formal presentations were desirable, rather discussion and exchange should be facilitated.

Dr. Gottesman felt the workshop should attempt to come up with some calculations. She said this had been done at the Pasadena workshop and proved very useful and helpful.

Dr. Gottesman pointed out that traditional breeding technologies can introduce genes from distant species into plants; for example, mammalian genes cannot be introduced into plants through traditional plant breeding but can be introduced using recombinant DNA techniques. She thought this topic might be worth discussing at the NIH-USDA workshop. She thought the conference should also address questions such as: How relevant are single gene changes in plants? Can gene movement or transfer into the environment occur? If it occurred, would this be a serious concern?

Dr. Arntzen wondered if this meeting should be geared to a particular audience. Dr. Miller said NIH workshops were held to help the RAC in its deliberations. The conclusions, however, have been used by scientific specialists, by the legal profession, by government policy-makers, by the press, and by the public. He did not feel the meeting should be geared to any particular audience.

Dr. Tolin asked if the working group thought the scope of the workshop should be limited to plants. The working group agreed that the scope should include plants and plant associated microorganisms. Dr. Tolin asked Dr. Betz of the EPA Office of Pesticides Programs (OPP) if examples of biorational pesticide use would help facilitate understanding. Dr. Betz agreed that use of microbial pest control agents could be discussed in the workshop program.

Dr. Arntzen suggested that an economist who could describe the impact of genetic technologies on agriculture should be invited to participate in the workshop. Dr. Fedoroff suggested that an individual with expertise in microbial ecology be invited to participate.

DEVELOPMENT OF GUIDANCE FOR EXPERIMENTS INVOLVING FIELD TESTING OF MICROORGANISMS

Dr. McGarrity asked the working group to begin discussing development of a guidance document for field testing of microorganisms.

Dr. Fedoroff suggested a microbial ecologist be added to the working group for this project. Dr. Vidaver pointed out that many current members of the working group have experience with plant pathogens and, thus, with microbial ecology. Dr. Gottesman agreed but pointed out that questions persist about the proposal to field test ice nucleation bacteria in spite of the fact that the modification is minor. She felt the mathematics of these types of procedures should be examined in detail to address questions such as: How many organisms are needed to impinge on an environment? How do numbers affect persistence and the ability to grow? How does number modulate the effects? She felt a specialist who could provide this type of expertise would be a valuable addition to the working group.

Dr. Gottesman felt RAC needed a document which would elucidate the parameters used to distinguish between trivial and non-trivial questions. The document should provide flexibility by providing guidance. Dr. Gottesman felt RAC and

its working groups should not be pressed into making a full scale review of trivial modifications.

Dr. Gottesman said experiments involving plants can be placed in either of two categories; the applications are either covered by Appendix L or must be reviewed on a case-by-case basis by RAC. She asked if such a distinction could be made for microorganisms.

Dr. McGarrity suggested a checklist might be generated. He said lawyers, who do not know the science, like to see checklists. Dr. Arntzen agreed that a checklist might be developed. He suggested proposals involving denitrifying bacteria might be approached in this way.

Dr. Gottesman said the working group should decide how specific a checklist should be; it will be difficult obtaining specific answers to all questions simply because all questions may not be equally applicable in all cases.

Dr. Fedoroff suggested data on water habitats as well as on soil habitats might be pertinent in a review of microorganisms.

Dr. Hollander called the attention of the working group to the EPA document distributed earlier in the meeting (Attachment III). Dr. Rissler said the document lists the questions which might be asked concerning the characteristics of organisms. She felt there is a limit to the number of questions which can be asked.

Dr. Fedoroff asked how the EPA document had been developed. Dr. Hollander replied that the document had been generated by EPA staff. Dr. Fedoroff asked Dr. Hollander if EPA has requested a group of scientific experts to review the document. Dr. Hollander replied that EPA has not yet requested expert review on this document but would do so. Dr. Hollander said she hoped the working group would offer EPA its evaluation of the document.

Dr. Fedoroff asked why EPA was soliciting working group evaluation of the document. Dr. Tolin said EPA has agreed to abide by the NIH Guidelines; EPA is abiding by that agreement in coming to RAC for RAC advice and aid in drafting EPA documents involving recombinant DNA.

Dr. Gottesman moved that each member the working group should respond individually to the EPA document; a subgroup of the working group would write a draft document concerning guidance for experiments involving field testing of microorganisms. The smaller group might use those portions of the EPA document which are useful. Dr. Arntzen seconded the motion. Dr. Scandalios asked the EPA representatives if they were comfortable with such a suggestion. They replied that they were comfortable with such a process. By a vote of twelve in favor, none opposed, and no abstentions, the working group accepted the motion.

Dr. Gottesman then moved to adjourn. Dr. Fedoroff seconded the motion, and it was unanimously approved. Dr. McGarrity adjourned the meeting at 3:15 p.m.

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Respectively submitted,

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William J. Gartland, Jr.
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10/5/84
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