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

RECOMBINANT DNA ADVISORY COMMITTEE  
SUBGROUP OF WORKING GROUP ON RELEASE INTO THE ENVIRONMENT  
MINUTES OF MEETING

SEPTEMBER 21, 1984

A subgroup of the Working Group on Release into the Environment met by telephone conference call from 3:45 p.m. to 4:15 p.m. on September 21, 1984, to attempt to develop a document dealing with points to consider in data submissions for experiments involving field testing of microorganisms. Drs. George Lacy, Thomas Pirone, Sue Tolin, Anne Vidaver, and Elizabeth Milewski participated.

Dr. Lacy introduced the document (Attachment I) which he and Dr. Tolin had generated on the basis of the September 14, 1984, telephone conference call. Drs. Vidaver and Pirone felt the document was well done; they had several minor suggested modifications.

Dr. Vidaver thought the general outline was good; she suggested that the word "characteristics" be substituted for the word "traits" wherever the word "traits" appears in the document.

Dr. Tolin asked if item II-C should explicitly state that information on toxogenicity be included in any proposal. She noted that this idea is implied in item II-C by the word "physiological." Drs. Vidaver and Pirone agreed that toxogenicity is implied in the term "physiological" and need not be stated explicitly in the document.

Dr. Pirone questioned the language of item III-A-4; he wondered if this language might be more logically included under item III-C-1. Dr. Lacy said item III-A-4 attempts to ask the following question: if the DNA construct consists of  $x + y$ , and  $x$  represents the gene, what is the function of the excess of DNA,  $y$ ? Would the presence of  $y$  affect the characteristics of the organism? Dr. Lacy suggested the word "excess" might be added to the language of item III-A-4 to clarify this concept.

Dr. Vidaver felt the language of item III-B-2 which contains a similar concept was not clear. Item II-B-2 reads as follows:

"Describe the amount of any vector DNA remaining in the final construction. Is any effect anticipated from this DNA?"

Dr. Lacy suggested that language might be modified to read as follows:

"Describe the amount of any vector DNA remaining in the final construction. Predict whether any changes in pathological, ecological, physiological, or

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genetic properties of the host organism would be anticipated as the result of any remaining vector DNA."

Dr. Vidaver noted that item III-B-3 should be reformatted.

Dr. Pirone suggested the word "likely" be deleted from item III-B-3-a-iii.

Dr. Vidaver suggested the words "pathological, ecological, physiological" be substituted for the words "pathologic, ecologic, physiologic" in items III-B-1, III-D, and IV-C-3.

Dr. Vidaver questioned the construction of item D-2. She suggested the language should read in part:

"Provide data related to any anticipated or non-anticipated effects of the modified microorganism from microcosm, greenhouse, and/or growth chamber experiments that simulate natural conditions. The methods of detection and sensitivity of sampling techniques, and periodicity of sampling should be indicated. These studies should include assessment of the following items:"

Dr. Lacy suggested item III-A-4 might more logically be inserted in this document as item D-2-d. Dr. Pirone agreed as population considerations would be important here. Dr. Lacy felt one consideration under item D-2 would be whether the DNA sequences to be introduced into the recipient organism might be adventitiously contaminated by other DNA sequences from the DNA donor organism. Dr. Tolin concerning the homogeneity of the DNA donor organism should be included in the document. Dr. Vidaver thought the question of the homogeneity of the donor might be addressed in item III-D.

Dr. Pirone noted that the phrase "including genetics" in items II-B and III-A-2 is very broad. Dr. Lacy said he had intended the phrase "including genetics" to encompass a request for information on the replication cycle. He noted that RAC would need to evaluate as part of its review information on the replication cycle of viruses and viroids. Dr. Vidaver suggested the qualifier "relevant" be included before the word "genetics" in items II-B and III-A-2.

Dr. Lacy wondered whether investigators submitting proposals would recognize that this request for relevant genetics would apply to RNA as well as DNA. Dr. Tolin questioned whether the investigator would include information on genetic variability and the stability of genomes in their proposals.

Dr. Lacy agreed that "relevant" might differ for different biological systems and agreed to insert this word in items II-B and III-A-2. Dr. Vidaver agreed; she felt no document could envisage every possibility.

Dr. Lacy questioned whether item IV-C-2 should be deleted. Dr. Tolin felt it should remain in the document as this section refers to the genetically modified organism.

Dr. Vidaver felt the language of item IV-E was not clear. Dr. Pirone agreed. Dr. Lacy said the language of item IV-E was an attempt to request information

on survival and dissemination. The working group suggested Dr. Milewski write language to convey the idea that the modified organism should be compared to the wild-type parent organism.

The document as modified by the subgroup is Attachment II. This document will be sent to the October 5, 1984, meeting of the Working Group in Release into the Environment.

Elizabeth Milewski  
Elizabeth Milewski, Ph.D.  
Executive Secretary

19 September 1984

Dr. Elizabeth Milewski  
Scientist Administrator  
Office of Recombinant DNA Activities  
National Institute of Allergy and  
Infectious Diseases  
Building 31, Room 310B  
Bethesda, MD 20205

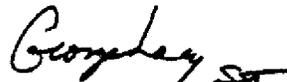
Dear Dr. Milewski:

Please please find enclosed a new version of the "points to consider for release of microorganism into the environment" based on our telephone conference of 14 September 1984.

Sincerely,



Sue A. Tolin  
Professor  
Plant Pathology



George H. Lacy  
Associate Professor  
Plant Pathology

cc:

Dr. T. P. Pirone  
Dr. A. K. Vidaver

Revisions by G. H. Lacy  
and S. A. Tolin based  
on A. Vidaver's draft  
of 30 August 1984  
September 18, 1984

Points to Consider for Submission Under Section III-A-2.

**Release of Microorganisms Containing Recombinant DNA Molecules into the Environment**

Experiments in this category require specific review by the RAC and NIH and IBC approval before initiation. Review of experiments under this category require submission of relevant information on the proposed experiments to ORDA for publication in the Federal Register and inclusion on the agenda of a RAC meeting.

The submission 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 background information and preliminary results conducted under containment should accompany the proposal. The following outline has been developed by the Working Group for Release into the Environment as a suggested list of Points to Consider for scientists preparing proposals. Information submitted should include but not be limited to:

- I. Summary of the experiment, including objectives, significance, and justification for the request.
- II. Characteristics of the parental microorganism to be modified and released into the environment
  - A. Identification, taxonomy, source, and strain
  - B. General biological characteristics, including replication cycle and genetics.
  - C. Pathological, ecological, and physiological traits
  - D. Methods for detection and monitoring natural populations of this microorganism. Give the sensitivity of these methods.
- III. Characteristics of the modified microorganism to be released into the environment
  - A. Source of the DNA sequences to be inserted into or used to modify the microorganism to be released into the environment.
    1. Identification, taxonomy, source and strain of organism donating the DNA

2. General biological characteristics, including genetics
  3. Pathological, ecological, and physiological traits
  4. Document the means and sensitivity of the techniques by which the presence or the absence of donor organism will be assessed in the final population of microorganisms to be released into the environment
- B. Genetic manipulations: describe the method(s) by which the DNA vector has been constructed and introduced. Include diagrams.
1. Describe the nature of the DNA inserted or modified by the genetic manipulations. Document the amount of any donor DNA in excess of the amount required for the minimal effective genetic alteration in the final construction. Predict whether any changes in pathologic, ecologic, physiologic or genetic properties of host organism would be anticipated as the result of the donor DNA.
  2. Describe the amount of any vector DNA remaining in the final construction. Is any effect anticipated from this DNA?
  3. Describe the method of introduction of the DNA and selection altered organisms.
    - a. If microorganisms are used to introduce vectors or are vectors themselves,
      - i. Indicate how they compare with wildtype strains.
      - ii. If disarmed pathogens are used to transmit the vector, indicate factors that will most likely prevent these microorganisms from regaining or acquiring pathogenic potential.
      - iii. If the vector is likely to survive independently of the host(s), refer to this possibility and provide any available data to assess the probability of transfer likely to other organisms
      - iv. If a microorganism will only be used to introduce a vector(s) or are vectors themselves, document the means and sensitivity of the techniques by which the presence or the absence of the microorganism will be assessed in the final construction to be released
- D. Properties of the modified organism
1. Describe anticipated properties of the final population of engineered organisms, with emphasis on the desired effect and any other possible pathologic, ecologic, physiologic or genetic effects.

2. Provide data related to any anticipated or non-anticipated effects of the modified microorganism from microcosm, greenhouse, and/or growth chamber experiments that simulate natural conditions. These studies should include assessment of the following items. The methods of detection and sensitivity of sampling techniques, and periodicity of sampling should be indicated.

- a. Survival of the microorganism to be released
- b. Replication of the microorganism to be released
- c. Dissemination routes of the microorganism

IV. Release into the environment

- A. Describe the experiment for release of the modified organism into the environment. Provide information, including diagrams, of the experimental location and the surrounding environs. Information provided should include pertinent plant, soil, water, aerobiologic, and/or other data to assess the containment or spread of the microorganism.
- B. Indicate containment procedures in the event of accidental release as well as intentional release, and procedures for emergency termination of the experiment. Specify access and security measures for the area(s) in which the tests will be performed
- C. If the microorganism to be released is to benefit a plant or control another microorganism, a noxious weed, or an animal pest of plants, provide the following information about that target organism:
  1. Identification, taxonomy, source, and strain
  2. Genetic characteristics
  3. Pathologic, ecologic, and physiologic traits
  4. The anticipated mechanism of the interaction between the released microorganism and the target organism
- D. Describe the prospective effects of the introduced microorganism on non-target plants, animals, and inanimate objects should be indicated as appropriate
- E. Describe monitoring procedures and their limits of detection for survival, dissemination, and non-target interactions of the modified microorganism. Include periodicity of sampling and rationales for monitoring procedures. Compare data collected with effects of the non-modified wildtype microorganism most similar to the modified organism at the experimental site.



DEPARTMENT OF HEALTH & HUMAN SERVICES

Attachment II - Page 1

Public Health Service

National Institutes of Health  
Bethesda, Maryland 20205  
Building : 31  
Room : 3B10  
(301) 496- 6051

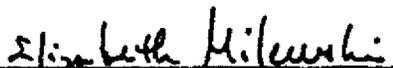
September 26, 1984

MEMORANDUM

To: Working Group on Release into the Environment  
From: Executive Secretary  
Subject: Additional Materials for October 5, 1984, Meeting

Please find enclosed a working preliminary paper which will hopefully serve as the seminal document for our October 5 attempt to develop submission guidance for investigators wishing to field test genetically modified microorganisms.

If you have any questions, please feel free to contact me at 301-496-6051.

  
Elizabeth Milewski, Ph.D.

Enclosure

Addresses: Dr. Arntzen  
Dr. Clowes  
Dr. Colwell  
Dr. Fedoroff  
Dr. Fowle  
Dr. Gottesman  
Dr. Hirano  
Dr. Lacy  
Dr. McGarrity  
Dr. Miller  
Dr. Mitchell  
Dr. Pimentel  
Dr. Pirone  
Dr. Pramer  
Dr. Scandalios  
Dr. Sharples  
Dr. Tolin  
Dr. Vidaver

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September 21, 1984

**POINTS TO CONSIDER FOR SUBMISSIONS INVOLVING RELEASE OF MICROORGANISMS  
CONTAINING RECOMBINANT DNA MOLECULES INTO THE ENVIRONMENT**

Experiments in this category require specific review and approval before initiation by the Recombinant DNA Advisory Committee (RAC) and the National Institutes of Health (NIH) and the Institutional Biosafety Committee (IBC). Relevant information on the proposed experiments should be submitted to the Office of Recombinant DNA Activities (ORDA).

The submission 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 background information and preliminary results conducted under containment should accompany the proposal. The following outline has been developed by the Working Group for Release into the Environment as a suggested list of Points to Consider for scientists preparing proposals. Information submitted should include but not be limited to:

- I. Summary of the experiment including objectives, significance, and justification for the request.
- II. Characteristics of the parental microorganism to be modified and released into the environment.
  - A. Identification, taxonomy, source, and strain.
  - B. General biological characteristics including replication cycle and relevant genetics.

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- C. Pathological, ecological, and physiological characteristics.
  - D. Methods for detection and monitoring natural populations of this microorganism. Describe the sensitivity of these methods.
- III. Characteristics of the modified microorganism to be released into the environment.
- A. Source of the DNA sequences to be inserted into or used to modify the microorganism to be released into the environment.
    - 1. Identification, taxonomy, source, and strain of organism donating the DNA.
    - 2. General biological characteristics including relevant genetics.
    - 3. Pathological, ecological, and physiological characteristics.
  - B. Genetic manipulations: describe the method(s) by which the DNA vector has been constructed and introduced. Include diagrams as appropriate.
    - 1. Describe the nature of the DNA inserted or modified by the genetic manipulations. Document the amount of any donor DNA in excess of the amount required for the minimal effective genetic alteration in the final construction. Predict whether any changes in pathological, ecological, physiological, or genetic properties of host organism would be anticipated as the result of the excess donor DNA.
    - 2. Describe the amount of any vector DNA remaining in the final construction. Predict whether any changes in pathological,

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ecological, physiological, or genetic properties of the host organism would be anticipated as the result of any remaining vector DNA.

3. Describe the method of introduction of the DNA and selection of altered organisms. If microorganisms are used to introduce vectors or are vectors themselves:
  - a. Indicate how they compare with wild-type strains;
  - b. If disarmed pathogens are used to transmit the vector, indicate factors that will most likely prevent these microorganism from regaining or acquiring pathogenic potential;
  - c. If the vector is likely to survive independantly of the host(s), refer to this possibility and provide any available data to assess the probability of transfer to other organisms;
  - d. If a microorganism will only be used to introduce a vector(s) or are vectors themselves, document the means and sensitivity of the techniques by which the presence or the absence of the microorganism will be assessed in the final construction to be released.

C. Properties of the modified organism.

1. Describe anticipated properties of the final population of engineered organisms with emphasis on the desired effect and any other possible pathological, ecological, physiological, or genetic effects.

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2. Provide data related to any anticipated or non-anticipated effects of the modified microorganism from microcosm, greenhouse, and/or growth chamber experiments that simulate natural conditions. The methods of detection and sensitivity of sampling techniques and periodicity of sampling should be indicated. These studies should include assessment of the following items.
  - a. Survival of the microorganism to be released.
  - b. Replication of the microorganism to be released.
  - c. Dissemination routes of the microorganism.
3. Document the means and sensitivity of the techniques by which the presence or the absence of donor organism will be assessed in the final population of microorganisms to be released into the environment.

IV. Release into the environment.

- A. Describe the experiment involving release of the modified organism into the environment. Provide information, including diagrams, of the experimental location and the surrounding environs. Information provided should include pertinent plant, soil, water, aerobiologic, and/or other data to assess the containment or spread of the microorganism.
- B. Indicate containment procedures in the event of accidental release as well as intentional release and procedures for emergency termination of 92

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the experiment. Specify access and security measures for the area(s) in which the tests will be performed.

- C. If the microorganism to be released is to benefit a plant or control another microorganism, a noxious weed, or an animal pest or plants, provide the following information about that target organism:
1. Identification, taxonomy, source, and strain;
  2. Genetic characteristics;
  3. Pathological, ecological, and physiological characteristics;
  4. The anticipated mechanism of the interaction between the released microorganism and the target organism.
- D. The prospective effects of the introduced microorganism on non-target plants, animals, and inanimate objects should be described as appropriate.
- E. Describe monitoring procedures and their limits of detection for survival, dissemination, and non-target interactions of the modified microorganism. Include periodicity of sampling and rationale for monitoring procedures. Collect data to compare the modified organisms with the non-modified wild-type microorganism most similar to the modified organism.