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

RECOMBINANT DNA ADVISORY COMMITTEE
WORKING GROUP ON DEFINITIONS

MINUTES OF MEETING¹

DECEMBER 5, 1986

The Working Group on Definitions of the Recombinant DNA Advisory Committee was convened at 9:00 a.m. on December 5, 1986, at the Marriott Hotel, Kenwood Room, 5151 Pooks Hill Road, Bethesda, Maryland, 20814. Dr. Gerard McGarrity was Chair. The following were present for all or part of the meeting:

Working Group members:

Susan Gottesman
Susan Hirano
Irving Johnson
Edward Korwek
Myron Levine

Gerard McGarrity
Paul Neiman
Thomas Pirone
David Pramer
Monica Riley

John Scandalios
Frances Sharples
Anne Vidaver
William Gartland
(Executive Secretary)

A working group roster is attached (Attachment I).

Other National Institutes of Health staff:

Stanley Barban, NIAID

Others:

M. Bradley Flynn, Department of Agriculture
Charles J. Eby, Monsanto Company
Alan Goldhammer, Industrial Biotechnology Association
James Kaper, University of Maryland, Baltimore
Elizabeth Milewski, Environmental Protection Agency
Henry Miller, Food and Drug Administration
David Moore, Association of American Medical Colleges
Greg Pearson, Blue Sheet
George Shibley, Department of Agriculture
Janet Shoemaker, American Society for Microbiology
Michael A. Swit, Burditt, Bowles & Radzius, Chartered
William Szkrybalo, Pharmaceutical Manufacturers Association
Sue Tolin, Department of Agriculture
L. F. Wright, Pfizer, Inc.

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

I. Definition of Deliberate Release.

Dr. McGarrity called the meeting to order and asked the observers to introduce themselves. He restated the charge to the working group and summarized the actions taken at the September 5, 1986, meeting of the working group. He noted that the Recombinant DNA Advisory Committee (RAC) had recommended approval of Dr. Gottesman's proposed amendment of Section III-A-2 of the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules at its meeting on September 29, 1986. However, the NIH Director has not yet acted on this recommendation. The RAC referred the other recommendations back to the working group for further consideration.

Dr. Gartland summarized a meeting on environmental release issues sponsored by the National Research Council at Millwood, VA, on October 27-28, 1986. He also summarized the conclusions and distributed a copy of the Report of the Committee to Review Allegations of Violations of the NIH Guidelines for Research Involving Recombinant DNA Molecules in the Conduct of Field Tests of a Pseudorabies Vaccine at Baylor College of Medicine and/or Texas A&M University. In response to a question, Dr. Milewski of the Environmental Protection Agency (EPA) stated that the purpose of the meeting of the EPA Biotechnology Science Advisory Committee subcommittee on environmental release on December 11-12, 1986, is to prepare several options for the definitions of "deliberate release" for use in EPA rulemaking procedures. The working group agreed to focus on matters pertaining to NIH and leave integration of agency decisions to the Biotechnology Science Coordinating Committee.

Dr. Vidaver then suggested that a second sentence be added to the definition of "deliberate release" which she had proposed at the September 5, 1986, meeting. The two sentences to be added to Section III-A-2 would read as follows:

"The term 'deliberate release' is defined as a planned introduction of recombinant DNA-containing microorganisms, plants, or animals into the environment. This is the experimental use of microorganisms, plants, or animals under conditions considered to be accepted scientific practice."

The appendices to be developed would incorporate the accepted practices. Drs. Korwek and Gottesman questioned how unplanned introductions would be treated if this definition is adopted. Drs. McGarrity and Vidaver pointed out that these sentences would be added to Section III-A-2, and the heading of Section III-A, "Experiments That Require RAC Review and NIH and IBC Approval Before Initiation," indicates that Section III-A-2 applies to experimental releases. Dr. McGarrity said that these sentences would presumably be added to the end of Dr. Gottesman's proposed revision of Section III-A-2.

Dr. Sharples then proposed an alternative rewrite of Section III-A-2 as follows:

"III-A-2. Environmental applications conducted without physical [and biological] containment of any organism containing recombinant DNA, except:

- "a. Certain plants as described in Appendix L.
- "b. Deletion derivatives not otherwise covered by these Guidelines.
- "c. Organisms covered in exemption III-D-2."

Dr. Johnson then moved that the two sentences of Dr. Vidaver be added at the end of the proposed further revision of Section III-A-2. After further discussion, Dr. Johnson amended his motion and moved adoption of Dr. Vidaver's first sentence for inclusion in Section III-A-2. The working group accepted the motion by a vote of 11 in favor, none opposed, and 1 abstention.

Dr. Gottesman then moved that Dr. Vidaver's second sentence be placed under "a" and that Section III-A-2 be revised to read in its entirety as follows:

"III-A-2. Deliberate release into the environment of any organism containing recombinant DNA except those listed below. The term 'deliberate release' is defined as a planned introduction of recombinant DNA-containing microorganisms, plants, or animals into the environment.

- "a. Introductions conducted under conditions considered to be accepted scientific practices in which there is adequate evidence of biological and/or physical control of the recombinant DNA-containing organisms. The nature of such evidence is described in Appendices L, M, N, and O.
- "b. Deletion derivatives not otherwise covered by these Guidelines.
- "c. Organisms covered in exemption III-D-2."

Dr. Johnson seconded the motion and the working group passed the motion by a vote of 10 in favor, 1 opposed, and 1 abstention.

Dr. Tolin stated that the U.S. Department of Agriculture will be proposing material for inclusion in Appendices L, M, and N.

II. Vaccine Development.

Dr. Levine presented information to the working group on development of several different varieties of live bacterial vaccines (Attachment II). He said that phase 1 studies of vaccines made by recombinant DNA techniques can be carried out in very closed facilities, but that mechanisms are needed to permit phase 2 and 3 clinical trials which may involve thousands of individuals. He pointed out that non-recombinant live attenuated vaccines are tested with no special constraints and predicted that superior and more precise vaccines will be made by recombinant DNA techniques.

Dr. Gottesman pointed out that there are already procedures in the NIH Guidelines for approval by other Federal agencies of experiments falling under Section III-A. Presumably these clinical trials would be submitted to the Food and Drug Administration under Investigational New Drug (IND) procedures. Dr. Korwek pointed out that there could be a problem with pre-human testing in animals since an IND is not required at this stage. He said this could be addressed in an appendix to the NIH Guidelines.

Dr. Gottesman then moved that: (1) investigators in the field of vaccine development be apprised of the options for exemption from RAC review as specified in paragraph two of Section III-A, and (2) that a working group be organized to develop criteria and procedures for inclusion in an Appendix O (Vaccines) of Section III-A-2. The motion passed by a vote of 11 in favor, none opposed, and no abstentions. It was the sense of the working group that Appendix O cover vaccines, and that Appendix N cover microorganisms other than vaccines.

III. Definition of Recombinant DNA.

In response to a question by Dr. Neiman, Dr. Gartland summarized why the working group had been asked to consider the definition of "recombinant DNA." Dr. Korwek questioned the reasons for reconsidering this definition. Dr. Gottesman said that she is not aware that the definition needs to be changed to take into account any specific experiments. Dr. Korwek said that since Section III-A-2 of the NIH Guidelines will presumably be revised to handle deletion derivatives, he favored not changing the basic definition of recombinant DNA.

Dr. Riley said she felt it is important to exclude some things from the definition. She then moved the following amendment of a sentence proposed by Dr. Landy for inclusion in Section I-B at the September 5, 1986, working group meeting:

"Genomes which contain only deletions, duplications, transpositions, single-base changes, or rearrangements are not considered to be recombinant DNA irrespective of the method by which they were produced. Products of translocations within genomes are considered to be recombinant DNA."

Dr. Riley said that this wording would make a distinction between transpositions and translocations which had not been made earlier.

Dr. Korwek questioned why these concerns could not be handled as exemptions. Dr. Gottesman noted that these types of experiments are already exempt in the laboratory. An alternative approach to achieve the same end would be to reword "b" and "c" in a revised Section III-A-2.

Dr. Neiman said that these concepts about deletions, etc., pertain particularly to microorganisms, but he did not feel that this revision of the definition would be generally accepted by those in the scientific community who deal with more complex organisms with more stable genomes.

Dr. Gottesman noted that deletions, etc., are already exempt in Section III-D unless they also fall under Section III-A. An alternative approach would be to broaden "b" and "c" in the revised Section III-A-2.

Dr. Vidaver suggested that the word "foreign" be added to the current definition in (i) in Section I-B. It was pointed out that "foreign" would have to be defined in a footnote.

Dr. Riley withdrew her motion, and Dr. Gottesman then moved that the following possible changes in the definition of recombinant DNA be presented to the RAC for consideration:

1. The first paragraph of Section I-B would be revised to read as follows (new words in underlined):

"In the context of these Guidelines, recombinant DNA molecules are defined as either: (i) molecules which are constructed outside living cells by joining foreign natural or foreign synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) DNA molecules that result from the replication of those described in (i) above.

2. The following new footnote would be added:

"Rearrangements involving the introduction of DNA from different organisms or different strains of an organism will be considered recombinant DNA. Deletions, single-base changes, and rearrangements within a single genome will not involve the introduction of foreign DNA and therefore would not be considered recombinant DNA."

Several members expressed reservations about changing the definition of recombinant DNA and the rationale for such a fundamental change in the NIH Guidelines. The vote on the motion was 5 in favor, 2 opposed, and 3 abstentions.

The working group then voted on the proposal itself, i.e., on the desirability of making these proposed changes in Section I-B and the addition of a footnote. The vote was 2 in favor, 5 opposed, and 3 abstentions.

After further discussion, Dr. Gottesman moved the following:

"The working group agreed with the concept that certain types of recombinant DNA experiments which do not involve the introduction of foreign DNA need not be subjected to special regulation as 'recombinant DNA.' The working group were split as to whether they preferred dealing with this problem by changing the definition of recombinant DNA or by further modifications of the exemptions (e.g., those in III-A-2).²

²Executive Secretary's Note: The latter part of this sentence was changed by NIH staff to read: "...or by further modifications of other sections of the Guidelines (e.g., those in III-A-2)." in the version published for comment in the Federal Register of December 19, 1986 (51 FR 45650).

Therefore, the working group presents the following two options for public comment and RAC consideration:

"1. Change definition of recombinant DNA:

"The first paragraph of Section I-B would be revised to read as follows (new words underlined):

"In the context of these Guidelines, recombinant DNA molecules are defined as either: (i) molecules which are constructed outside living cells by joining foreign natural or foreign synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) DNA molecules that result from the replication of those described in (i) above.

"The following new footnote would be added at the word 'foreign':

"Rearrangements involving the introduction of DNA from different organisms or different strains of an organism will be considered recombinant DNA. Deletions, single-base changes and rearrangements within a single genome will not involve the introduction of foreign DNA and therefore would not be considered recombinant DNA."

"2. Modify Section III-A-2 to read as follows:

"III-A-2. Deliberate release into the environment of any organism containing recombinant DNA except those listed below. The term 'deliberate release' is defined as a planned introduction of recombinant DNA-containing microorganisms, plants, or animals into the environment.

- "a. Introductions conducted under conditions considered to be accepted scientific practices in which there is adequate evidence of biological and/or physical control of the recombinant DNA-containing organisms. The nature of such evidence is described in Appendices L, M, N, and O.
- "b. Deletion derivatives and single base changes not otherwise covered by the Guidelines.
- "c. Rearrangements and amplification within a single genome. Rearrangements involving the introduction of DNA from different strains of the same organism would not be covered by this exemption."

After voting 9 in favor, 1 opposed, and no absentions on the first sentence of the motion, the working group voted 9 in favor, none opposed, and 1

abstention that these options be published for comment in the Federal Register and considered by the RAC.

The working group members then voted on their preference for Option 1 or 2. The vote for Option 1, i.e., a change in the definition of recombinant DNA was 2 in favor, 7 opposed, and 1 abstention. The vote for Option 2, i.e., modification of Section III-A-2, was 6 in favor, 2 opposed, and 2 abstentions.

Dr. Neiman then moved that the vote taken earlier in the day on what is now Option 1 be superseded by the vote on the proposal to publish for comment and present to the RAC both Options 1 and 2. The vote was 9 in favor, none opposed, and one abstention.

IV. Adjournment.

The meeting of the working group was adjourned at 3:40 p.m.

Respectfully submitted,

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

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

Date

Gerard J. McGarrity, Ph.D.
Chair
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RAC SUBCOMMITTEE MEETING ON DEFINITIONS

Description of Varieties of Live Bacterial Vaccines that Should be Exempt
from Guidelines and from Restrictions on "Deliberate Release"

The application of modern biotechnology to vaccine development during the past five years has resulted in the appearance of many candidate vaccines. These include improved vaccines against diseases for which immunizing agents already exist as well as new vaccines against diseases which were heretofore without immunoprophylactic control measures. Many of the vaccines reaching the point of clinical trials consist of live, attenuated genetically-engineered bacteria, modified by means of recombinant DNA technology. Certain of the live bacterial vaccines for human and veterinary use that are prepared by recombinant DNA technology should be exempt from the Guidelines and should not require RAC approval or environmental impact statements from federal agencies prior to initiating clinical studies. These varieties of vaccines are reviewed below, along with suggestions for certain characteristics that the strains should possess.

1) "Self-Destructing" Bacterial Vaccines

One method of attenuating enteric bacterial pathogens is by means of modifying the production of certain enzymes in the Leloir pathway. As a consequence, grown in the presence of certain substrates, the mutant

bacteria autolyze as a result of the accumulation of intermediate products of metabolism that cannot be further processed. The best examples of this prototype bacterial vaccine are the attenuated Salmonella typhi and Salmonella typhimurium gal E mutant strains that have a complete lack of the enzyme UDP-galactose-4-epimerase. Ty21a, a galE mutant of S. typhi isolated in the early 1970s after chemical mutagenesis, has shown the advantages of this variety of attenuation. Grown in the presence of galactose, which results in the production of smooth lipopolysaccharide O antigen, this vaccine strain is safe, immunogenic and protective but is also rarely recoverable from coprocultures. Large-scale field trials in Egypt and Chile, involving more than 600,000 schoolchildren, have demonstrated the safety and efficacy of the Ty21a live oral typhoid vaccine. Ty21a is presently licensed in many countries of Europe, Latin America, Asia and Africa and is expected to be licensed shortly in the U.S.A. Following ingestion of doses of this live oral vaccine containing circa 1-3 billion viable organisms, the vaccine is not recoverable from coprocultures. This is a consequence of the method of attenuation.

GalE mutants of S. typhi, S. typhimurium, and Shigella flexneri have been prepared by recombinant DNA techniques, by means of deletions of the gal E gene. These vaccines have distinct potential advantages over chemically mutagenized strains and clinical evaluations of the safety and immunogenicity of these vaccine candidates should therefore be expedited.

Self-destructing, non-transmissible vaccine strains of the above variety should be exempt from the guidelines. It should be recommended, however, that vaccine candidates of this variety should contain a marker such as a resistance to Hg⁺⁺ ions, a stable biochemical marker, or resistance to a clinically irrelevant antibiotic, to allow ready

identification of the vaccine strain and its differentiation from wild type strains.

2) Auxotrophic Strains

Another approach by which bacterial pathogens may be suitably attenuated to serve as live vaccine strains is to render them auxotrophic for substrates that are unavailable in the human or animal tissues or body fluids. The best examples of this variety of attenuation are the Aro- derivatives of S. typhi and S. typhimurium. These mutants have deletions of the Aro A gene rendering them unable to persist in the mammalian body because of the lack of 2,3, dihydroxybenzoate. As a consequence these attenuated mutants cannot proliferate to reach high numbers in the mammalian host and cause disease but they persist sufficiently long to stimulate immune responses. Aro- mutants of S. typhimurium have been shown to be safe and protective vaccines in mice and cattle, while the safety and immunogenicity of an Aro-, Pur- S. typhi vaccine strain (541Ty) has recently been demonstrated in Phase 1 clinical studies in man.

Auxotrophic mutants can be prepared by recombinant DNA technology, as well as by the classical genetic techniques (using phages to create the deletions) employed to prepare 541Ty. These mutants should possess some stable marker allowing them to be clearly discernable from wild type organisms.

3) Proven Attenuated Bacteria Acting as "Carrier" Strains to Express Foreign Genes of other Organisms

Attenuated S. typhi strain Ty21a, because of its record of safety and its stimulation of both cell-mediated as well as humoral immune responses,

is being used to carry and express cloned genes of critical, putatively protective antigens of other organisms. For example, modified Ty21a expressing the plasmid-encoded O antigen of S. sonnei and Ty21a expressing the cloned genes for *Vibrio cholerae* O1 serotype Inaba have been prepared. Known attenuated strains, such as Ty21a, carrying cloned genes from other organisms should be excluded from the guidelines, as long as the introduced genes do not encode a potent holotoxin.

4) Strains with Deletions of Chromosomal Genes Encoding Critical Virulence Properties

For some bacterial pathogens, a chromosomal gene product is an absolute necessity for full expression of pathogenicity. One such example is V. cholerae O1. The severe diarrheal purging characteristic of cholera gravis is the consequence of the effects of cholera enterotoxin which consists of five B (binding) subunits and one A (biologically active, ADP-ribosylating) subunit. Ingestion of minute amounts (5 mcg) of purified cholera enterotoxin can result in severe purging. Similarly, deletion of the genes encoding the A subunit renders the mutant unable to cause cholera gravis. An example of such a vaccine strain is CVD 103, a genetically-engineered A-B+ mutant of a V. cholerae classical Inaba strain. CVD 103 does not cause severe diarrhea, is highly immunogenic and is highly protective.

Live vaccines attenuated by the deletion of critical virulence properties should also be exempt, as long as they have a stable marker to differentiate them from wild type strains and particularly if they have a further mutation in the rec A gene. The latter defect virtually assures that DNA introduced by conjugation will not be incorporated into the

vaccine genome.

5) Vaccine Strains Expressing CRM Toxoids

Another approach is to modify the toxin genes of organisms in which toxin is the critical virulence property and where antitoxin is important in protection so that the mutant elaborates a biologically inactive albeit immunogenic toxoid molecule (so-called cross-reacting molecule or CRM). Such mutants should have stable markers and should ideally be rec A minus strains or their equivalent.

6) Bacteria with Plasmids Having Deletions of Critical Virulence Genes

For some bacteria the critical virulence genes are plasmid-encoded and often two distinct genes (for example encoding ST and colonization fimbriae) are adjacent. Vaccine strains containing plasmids having deletions of critical virulence genes should also be exempt from the guidelines.