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Part IV

Department of Health, Education, and Welfare

National Institutes of Health

Guidelines for Research Involving Recombinant DNA Molecules

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

National Institutes of Health

Recombinant DNA Research; Actions Under Guidelines

AGENCY: National Institutes of Health.

ACTION: Notice of actions under NIH Guidelines for Research Involving Recombinant DNA Molecules.

SUMMARY: This notice sets forth actions taken by the Director, NIH under the 1978 NIH Guidelines for Research Involving Recombinant DNA Molecules (43 FR 60108).

EFFECTIVE DATE: July 20, 1979.

FOR FURTHER INFORMATION CONTACT: Additional information can be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20205, (301) 496–6051.

SUPPLEMENTARY INFORMATION: I am promulgating today several major actions under the NIH Guidelines for Research Involving Recombinant DNA Molecules. These proposed actions were published for comment in the **Federal Register** of April 13, 1979 and reviewed and recommended for approval by the Recombinant DNA Advisory Committee (RAC) at its meeting on May 21, 22, and 23, 1979. In accordance with Section IV-E-1-b of the NIH Guidelines, I find that these actions comply with the Guidelines and present no significant risk to health or the environment.

Part I of this announcement provides background information on the actions. Part II provides a summary of the major actions.

I. Decisions on Actions Under Guidelines

A. Containment levels for certified *Saccharomyces Cerevisiae* and *Neurospora Crassa* HV systems

The RAC at its February 15–16, 1979 meeting recommended the use of *Saccharomyces cerevisiae* and *Neurospora crassa* as HV1 systems and specified certain strains and vectors of *S. cerevisiae* as HV2 host-vector systems. The certified systems were to be used as follows:

"In accord with Section III–C–5, host-vector systems which have been approved as HV1 systems may be used under P2 containment conditions for shotgun experiments with phages, plasmids, and DNA from nonpathogenic prokaryotes which do not produce polypeptide toxins [34]. For other classes of recombinant DNA experiments with these HV1 systems, except for the cloning of complete genomes of eukaryote viruses, the *S. cerevisiae* and *N. crassa* HV1 systems and *S. cerevisiae* HV2 systems may be used at the physical containment levels applicable to EK1 and EK2 systems, respectively."

While the RAC generally approved equivalence between these HV systems and the EK designations in the Guidelines, there was concern raised about the appropriate levels of containment when complete genomes of eukaryotic viruses are cloned into these organisms.

Prior to the RAC considering this issue at it February 15–16, 1979 meeting, it was mistakenly printed in the **Federal Register** of January 15, 1979 as follows:

"Experiments involving complete genomes of class 1 eukaryote viruses will require P3 + HV2 containment levels. Other eukaryote viruses are to be handled on a case-by-case basis [45]."

The correct wording should have been "will require P3 + HV1 or P2 + HV2 containment levels."

The RAC in its deliberations at the February 15–16, 1979 meeting took a less restricted position and recommended that the following wording be substituted for the previous proposal:

"Experiments involving complete genomes of eukaryote viruses will require P3 + HV1 or P2 + HV2 containment levels."

This recommendation passed by a vote of 17 to 1 with 2 abstentions. Since this was a major change in the proposed action as it appeared in the **Federal Register** on January 15, 1979, additional opportunity for public comment was deemed appropriate. Accordingly, this proposal was published in the **Federal Register** on April 13, 1979 for an additional 30-day comment period prior to its presentation at the May 21–23, 1979 RAC meeting. During the 30-day comment period no comments were received.

Based on the containment features of these organisms which were previously presented in reports to the RAC and the discussion at the February 15–16, 1979 meeting, the RAC at its May, 1979 meeting voted 17 to 0 with 2 abstentions to accept this proposal.

B. Containment levels for unmodified laboratory strains of Neurospora Crassa

Based on extensive analysis of the fungus *N. crassa*, the NIH has previously approved genetically modified strains of this organism as HV1 as stated in the **Federal Register** of April 11, 1979. At its February 15–16, 1979 meeting, the RAC also recommended a limited use of unmodified laboratory strains of *N. crassa*. The NIH accepted the following conservative interpretation of the RAC's position based on the discussion at the time and until there was opportunity for further clarification by the RAC:

"Unmodified laboratory strains of *N. crassa* are approved at the P3 level of containment for shotgun experiments with phages, plasmids, and DNA from Class 1 prokaryotes [1] and lower eukaryotes that do not produce polypeptide toxins [34]."

Based on the need for further clarification, the following alternate interpretation of the RAC's action was published in the **Federal Register** on April 13, 1979 for an additional 30-day comment period prior to its consideration at the May 21–23, 1979 meeting. During the 30-day comment period no comments were received on this proposal:

"Unmodified laboratory strains of *Neurospora crassa* can be used in all experiments for which HV1 *N. crassa* systems are approved provided that these are carried out at physical containment one level higher than required for HV1. However, if P3 containment is specified for HV1 *N. crassa*, this level is considered adequate for unmodified *N. crassa*. For P2 physical containment, special care must be exercised to prevent aerial dispersal of macroconidia, including the use of a biological safety cabinet."

The discussion on this issue at the May, 1979 RAC meeting followed that which has been described in the **Federal Register** of April 11, 1979. Essentially, there was concern about the escape of *N. crassa* since it forms spores which are freely dispersed. As a result of this concern, it was recommended at the February, 1979 meeting that all experiments with wild type *N. crassa* should require the use of a biological safety cabinet. At the May 21–23, 1979 meeting of the RAC, it was pointed out that the organism has ony a small ecological niche and that it is a nonpathogenic organism.

The RAC accepted the use of unmodified laboratory strains of *N. crassa* as published in the **Federal Register** of April 13, 1979, by a vote of 11 to 2 with 5 abstentions. It was the sense of the RAC that the principle of equivalency of HV systems with EK systems applies at the present time only to the setting of containment levels for shotgun experiments. It does not apply at the present time to lowering of containment levels for characterized or purified DNA preparations and clones, to returing DNA segments to non-HV1 host of origin, etc.

C.1 Transfer of cloned DNA segments to Eukaryotic organisms

Based on the recommendation of the RAC at its February 15–16, 1979 meeting, the NIH previously has approved the

return of DNA segments to a higher eukaryotic host of origin as stated in the **Federal Register** on April 11, 1979:

"III–C–6. *Return of DNA Segments to a Higher Eukaryotic Host of Origin.* DNA from a higher eukaryote (Host D) may be inserted into a lambdoid phage vector or into a vector from certified EK2 host-vector system and propagated in *E. coli* K–12 under the appropriate containment conditions [see Section III–A–1]. Subsequently, this recombinant DNA may be returned to Host D and propagated under conditions of physical containment comparable to P1 and appropriate to the organism under study [2A]."

Several commentators had requested that this section be broadened to permit the heterologous transfer of DNA segments to a eukaryote other than the host of origin. A broader proposal could not be considered at the February 15–16, 1979 RAC meeting as it would require the opportunity for public comment. The following proposed revision of Section III–C–6 was published in the **Federal Register** on April 13, 1979 for a 30-day comment period prior to its consideration at the May 21–23, 1979:

"III–C–6. *Transfer of Cloned DNA Segments to Eukaryotic Organisms.* DNA from any nonprohibited source [Section I–D] which has been cloned and propagated in *E. coli* under appropriate physical containment conditions, may be transferred with the *E. coli* vector used for cloning to a ekaryotic organism or cells in culture and propagated under conditions of physical containment comparable to P1 and appropriate to the organism under study [2A]."

Several letters were received during the comment period which supported this proposal. Recent experimental results have demonstrated that it is possible to transfer DNA to eukaryotic organisms without a requirement for the DNA to be part of a recombinant DNA molecule. The major difference in the interspecies experiments which involve using recombinant DNA techniques is that there will be an association of the *E. coli* vector DNA with the DNA of interest. The recombinant DNA methods also allow a more controlled process since it permits the use of selected genes.

A very important category of experiments which this amendment would permit involves the cloning of DNA from one higher eukaryote into *E. coli,* followed by the transfer to an embryo or teratoma of another eukaryote. This procedure will enable the study of the genetic basis of various diseases by isolating individual genes and examining their expression in various whole animals. There will be the possibility of understanding the basis for cell diversification during development of higher organisms and the organization of genetic information. These features may be important in many cases to understanding the origins of malignant growth and the genetic basis of disease. As noted by one commentator, this research may lead to the "possible cure of human genetic diseases."

One commentator indicated that this proposal would, in essence, allow for nearly any eukaryote to become a host for any DNA; this would not be in the spirit of the Guidelines. Another commentator noted that any experiments that involved the return of cloned DNA to humans would require the examination by human experimentation committees.

These comments were discussed at the May 21–23, 1979 RAC meeting, and concern was expressed over the broad nature of the proposal. The use of recombinant DNA methods for studying diseases using whole animals or plants was generally supported by the RAC. It was agreed that this revision should appear as a new section of the Guidelines, III–C–7. The following more restrictive amendment was proposed by the RAC to limit the experiments to easily contained whole organisms and to only small portions of viruses:

"III–C–7. *Transfer of Cloned DNA Segments to Eukaryotic Organisms.*

III–C–7–a. *Transfer to Non-human Vertebrates.* DNA from any nonprohibited source [Section I–D], except for greater than one quarter of a eukaryotic viral genome, which has been cloned and propagated in *E. coli* under appropriate physical containment conditions, may be transferred with the *E. coli* vector used for cloning to any eukaryotic cells in culture or to any non-human vertebrate organism and propagated under conditions of physical containment comparable to P1 and appropriate to the organism under study [2A]. Transfers to any other host will be considered by the RAC on a case-by-case basis [45].

III–C–7–b. *Transfer to Higher Plants.* DNA from any nonprohibited source [Section I–D] which has been cloned and propagated in *E. coli* under appropriate containment conditions, may be transferred with the *E. coli* vector used for cloning to any higher plant organisms (Angiosperms and Gymnosperms) and propagated under conditions of physical containment comparable to P1 and appropriate to the organism under study [2A]. Intact plants or propagative plant parts may be grown under P1 conditions described under Section III–C–3. Containment must be modified to ensure that the spread of pollen, seed or other propagules is prevented. This can be accomplished by conversion to negative pressure in the growth cabinet or greenhouse or by physical entrapment by "bagging" of reproductive structures. Transfers to any other plant organisms will be considered on a case-by-case basis [45]."

The RAC accepted proposal III–C–7–a by a vote of 19 to 2 with 2 abstentions, and proposal III–C–7–b by a vote of 18 to 0 with 1 abstention.

D. Proposed exemption under I–E–5 for cloning in tissue culture cells

The RAC considered a proposal for exempting experiments involving the propagation of recombinant DNA molecules from non-viral components in tissue culture cells. The proposal, made by Dr. Wallace Rowe, appeared in the April 13, 1977 **Federal Register** as follows:

"Those recombinant DNA molecules that are propagated in cells in tissue culture and that are derived entirely from non-viral components (that is, no component is derived from a eukaryotic virus) or that contain no more than one-fourth of the genome of a eukaryotic virus are exempt from the Guidelines."

During the 30-day period for comment, one comment was received on the proposed action. This commentator opposed the motion on the grounds that the introduction of recombinant DNA molecules linked even to only one-fourth of a viral genome in tissue culture cells may possibly generate altered endogenous or exogenous viruses in the cells.

The RAC considered this proposal following a discussion of its merits by Dr. Rowe. It was pointed out that tissue culture cells are well contained and safe systems for studying gene function. Some members of the RAC expressed concern that recombinant molecules containing one-fourth of a viral genome might possibly generate infectious virus. Dr. Rowe agreed to amend his proposal to delete the portion that referred to exempting recombinant molecules containing less than one-fourth of a eukaryotic viral genome.

The RAC voted 17 to 3 with 2 abstentions to accept the amended proposal with a minor modification in the wording to include *"and maintained"* in cells, as follows:

"Those recombinant DNA molecules that are propagated and maintained in cells in tissue culture and that are derived entirely from non-viral components (that is, no component is derived from a eukaryotic virus) are exempt from the Guidelines."

E. Containment levels for experiments involving Genera *Streptomyces* and *Micromonospora*

The RAC at its May 21–23, 1979 meeting considered a proposal that had been submitted by the Working Group on Prokaryotic Host-Vectors other than *E. coli* that would allow the formation of

recombinant molecules between members of the *Actinomycetes* group at P2 physical containment except for species which are known to be pathogenic for man, animal, or plants. The following notice appeared in the **Federal Register** on April 13, 1979:

"P2 physical containment shall be used for DNA recombinants produced between members of the *Actinomycetes* group except for those species which are known to be pathogenic for man, animals or plants [2A]."

During the 30-day comment period no comments were received. The discussion on this proposal was led by Dr. Julian Davies, University of Wisconsin, an *ad hoc* consultant at the RAC meeting on May 21, 1979. It was explained that the *Actinomycetes* are a large group of closely related organisms, many of which are used to produce therapeutically active compounds. Ninety percent of the antibiotics produced industrially are derived from the *Micromonospora* and *Streptomyces* genera. They are mainly soil organisms, and do not exist in the gut. Genetic exchanges occur in almost all cases for which it has been looked. The basis for exchange includes recombination, mating, and fusion (heterokaryosis). Plasmid transfer of genetic information has also been demonstrated. Substantial DNA homology from 20–80% has been demonstrated in the *Streptomyces* genus. Although some members of the *Actinomycetes* are known as pathogens for man, animals and plants, the *Streptomyces* and *Micromonospora* genera are *non-pathogenic* for man and animals. An extensive search of the literature has revealed no reports of pathogenicity. Based on the importance of these microorganisms, non-pathogenicity, and evidence for genetic relatedness, it was proposed that recombinant experiments between the *Streptomyces* and *Micromonospora* species be permitted under P2 containment. The original proposal was restricted by the RAC to only the *Streptomyces* and *Micromonospora* genera.

A motion to accept this proposal, amended as follows, was passed with 16 for, none opposed, and 2 abstentions:

"P2 physical containment shall be used for DNA recombinants produced between members of the genera *Streptomyces* and *Micromonospora* except for those species which are known to be pathogenic for man, animals or plants [2A]."

F. Exemption for streptomyces species that exchange genetic information

The Working Group on Prokaryotic Host-Vectors other than *E. coli* recommended that a list of *Streptomyces* species that have been shown to exchange chromosomal DNA be placed in the exemption category of Section I–E–4. This proposal was published in the **Federal Register** on April 13, 1979 as follows:

"*Streptomyces* species that have been shown to exchange chromosomal DNA are proposed to be included under the exemption category of Section I–E–4 of the 1978 Guidelines. Any recombinant DNA molecules that are composed entirely of DNA segments from one or more of the organisms listed below and to be propagated in any of the organisms listed below are exempt from the Guidelines. (This list is to be separate from the other lists of exempt organisms in Appendix A.)

Streptomyces aureofociens
Streptomyces rimosus
Streptomyces coelicolor
Streptomyces griseus
Streptomyces cyoneus
Streptomyces venezueloe."

During the 30-day comment period no comments were received. The RAC considered the criteria for genetic exchange that were set forth as a basis for placing a proposed list of *Streptomyces* species in the exemption category of section I–E–4 of the Guidelines. A motion that physiological heterokaryosis between intact organisms shall be taken as evidence of genetic exchange under criterion 2 in the discussion of Appendix A of the NIH Director's December 22, 1978 decision document was passed 18 to 0 with 1 abstention.

A motion was made to divide the list of the six proposed *Streptomyces* species into two sublists of three each because the evidence for pair-wise exchange was not as strong between the two sublists as the exchange within each sublist. The sublists are as follows:

Sublist 1

Streptomyces aureofaciens
Streptomyces rimosus
Streptomyces coelicolor

Sublist 2

Streptomyces griseus
Streptomyces cyaneus
Streptomyces venezueloe

Any recombinant DNA molecules that are composed entirely of DNA segments from one or more of the organisms within each sublist and to be propagated in any of the organisms included in that sublist are exempt from the Guidelines. (This list is to be separate from the other lists of exempt organisms in Appendix A.)

The motion was accepted by a vote of 14 for, none opposed, and 5 abstentions.

G. Use of *agrobacterium tumefaciens* as a host-vector system

Dr. Mary-Dell Chilton of the University of Washington submitted a proposal for approval of *Agrobacterium tumefaciens* and its Ti (tumor-inducing) plasmid as a host-vector system for recombinant DNA experiments. Crown gall tumors caused by *A. tumefaciens*, a ubiquitous inhabitant of the soil, are induced by tumor genes located on the large Ti plasmids. The Ti plasmid enters plant cells and inserts itself in the plant chormosomal DNA. The Ti plasmids appear promising as vectors for introduction of desired foreign DNA into higher plants.

Notice of this proposal was first published in the **Federal Register**, April 13, 1979 as follows:

"Non-disabled strains of *Agrobocterium tumefociens* can be used in combinations with the cointegrate plasmid Ti::RP4 as a host-vector system at the P3 level of physical containment."

No comments were received by the Office of Recombinant DNA Activities during the 30-day period following the publication of this proposal. On April 25, 1979, Dr. Chilton submitted a supplement to her original proposal which represented an alternative approach for using the *Agrobacterium* system that would provide greater biological containment. The new strategy was described by Dr. Chilton at the RAC meeting on May 21, 1979. First, eukaryotic DNA would be inserted in a non-conjugative plasmid, i.e. pBR322, that also contains fragments of Ti plasmid DNA and an insert of the origin of replication of other cryptic *Agrobacterium* plasmids. The plasmid would be propagated in *E. coli* K–12 and the recombinant DNA molecules used to transform *A. tumefaciens*. The *A. tumefaciens* host strain would then be employed to induce tumors in higher plants. The advantage of the newer strategy is that it avoids the involvement of RP4 which is a wide range conjugative replicon. Dr. Chilton proposed a one step higher level of containment required for the eukaryotic insert when the Ti plasmid is used. In the RAC discussion, it was pointed out that although the **Federal Register** notice of April 13, 1979 cited the use of the plasmid Ti::RP4, this new experimental approach was much safer. A two-part motion was considered by the RAC:

a. Approve the cloning of well-characterized fragments of eukaryotic DNA under P3 conditions, either in *E. coli* K–12 or in *A. tumefaciens* carrying a Ti plasmid, using an EK2 plasmid vector coupled to a fragment of the Ti plasmid and/or the origin of replication of a cryptic *A. Tumefaciens* plasmid.

b. Approve introducing the bacteria into plant parts or cells in culture under P3 containment conditions.

The motion was passed by the RAC by a vote of 14 for, 2 opposed, with 3 abstentions. It was noted that this recommendation is narrower and more restrictive than the proposal published for comment in the Federal Register of April 13, 1979. It was also noted that recommendation of this proposal should not be construed as a general approval of the *Agrobacterium* system as a new cloning system.

II. Summary of Major Actions Under Guidelines

A. Containment levels for certified *Saccharomyces cerevisiae* and *Neurospora crassa* HV systems

In accord with Section III–C–5, host-vector systems which have been approved as HV1 systems may be used under P2 containment conditions for shotgun experiments with phages, plasmids, and DNA from nonpathogenic prokaryotes which do not produce polypeptide toxins [34]. For other classes of recombinant DNA experiments with these HV1 systems, except for the cloning of complete genomes of eukaryote viruses the *S. cerevisiae* and *N. crassa* HV1 systems and *S. cerevisiae* HV2 systems may be used at the physical containment levels applicable to EK1 and EK2 systems, respectively. Experiments involving complete genomes of eukaryote viruses will require P3+HV1 or P2+HV2 containment levels.

B. Containment levels for unmodified laboratory strains of *Neurospora crassa*

Unmodified laboratory strains of *Neurospora crassa* can be used in all experiments for which HV1 *N. crassa* systems are approved provided that these are carried out at physical containment one level higher than required for HV1. However, if P3 containment is specified for HV1 *N. crassa*, this level is considered adequate for unmodified *N. crassa*. For P2 physical containment, special care must be exercised to prevent aerial dispersal of macroconidia, including the use of a biological safety cabinet.

C. III–C–7. Transfer of cloned DNA segments to Eukaryotic organisms

III–C–7–a. *Transfer to Non-human Vertebrates.* DNA from any nonprohibited source [Section I–D], except for greater than one quarter of a eukaryotic viral genome, which has been cloned and propagated in *E. coli* under appropriate physical containment conditions, may be transferred with the *E. coli* vector used for cloning to any eukaryotic cells in culture or to any non-human vertebrate organism and propagated under conditions of physical containment comparable to P1 and appropriate to the organism under study [2A]. Transfers to any other host will be considered by the RAC on a case-by-case basis [45].

III–C–7–b. *Transfer to Higher Plants.* DNA from any nonprohibited source [Section I–D] which has been cloned and propagated in *E. coli* under appropriate containment conditions, may be transferred with the *E. coli* vector used for cloning to any higher plant organisms (Angiosperms and Gymnosperms) and propagated under conditions of physical containment comparable to P1 and appropriate to the organism under study [2A]. Intact plants or propagative plant parts may be grown under P1 conditions described under Section III–C–3. Containment must be modified to ensure that the spread of pollen, seed or other propagules is prevented. This can be accomplished by conversion to negative pressure in the growth cabinet or greenhouse or by physical entrapment by "bagging" of reproductive structures. Transfers to any other plant organisms will be considered on a case-by-case basis [45].

D. Exemption under I–E–5 for cloning in tissue culture cells

In accord with Section I–E–5, those recombinant DNA molecules that are propagated and maintained in cells in tissue culture and that are derived entirely from non-viral components (that is, no component is derived from a eukaryotic virus) are exempt from the Guidelines.

E. Containment levels for experiments involving genera *Streptomyces* and *Micromonospora*

P2 physical containment shall be used for DNA recombinants produced between members of the genera *Streptomyces* and *Micromonospora* except for those species which are known to be pathogenic for man, animals or plants [2A].

F. Exemption for *Streptomyces* species that exchange genetic information

The following two sublists of *Streptomyces* species that have been shown to exchange chromosomal DNA are included under the exemption category of Section I–E–4 of the 1978 Guidelines. Any recombinant DNA molecules that are composed entirely of DNA segments from one or more of the organisms within each sublist and to be propagated in any of the organisms included in that sublist are exempt from the Guidelines. (This list is to be separate from the other lists of exempt organisms in Appendix A.)

Sublist 1

Streptomyces aureofaciens
Streptomyces rimosus
Streptomyces coelicolor

Sublist 2

Streptomyces griseus
Streptomyces cyaneus
Streptomyces venezuelae

G. Use of *Agrobacterium Tumefaciens* as a host-vector system

The NIH has approved the cloning of well-characterized fragments of eukaryotic DNA under P3 conditions, either in *E. coli* K–12 or in *A. tumefaciens* carrying a Ti plasmid, using an EK2 plasmid vector coupled to a fragment of the Ti plasmid and/or the origin of replication of a cryptic *A. tumefaciens* plasmid.

The NIH has approved introducing these bacteria into plant parts or cells in culture under P3 containment conditions.

Dated: July 13, 1979.

Donald S. Fredrickson,

Director, National Institutes of Health.

[FR Doc. 79–22446 Filed 7–19–79; 8:45 am]

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