Bioprocessing Safety

Worker and Community Safety and Health Considerations

Warren C. Hyer, Jr. editor

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Each paper published in this volume was evaluated by three peer reviewers. The authors addressed all of the reviewers' comments to the satisfaction of both the technical editor(s) and the ASTM Committee on Publications.

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Foreword

This publication, *Bioprocessing Safety: Worker and Community Safety and Health Considerations*, contains papers presented at the First International Symposium on Large-Scale Bioprocessing Safety: Worker and Community Safety and Health Considerations, which was held on 6–8 Oct. 1987 in Washington, DC. The symposium was sponsored by ASTM Committee E-48 on Biotechnology, in cooperation with the following organizations: American Biological Safety Association, American Industrial Hygiene Association, American Society for Microbiology, Association of Biotechnology Companies, Biotechnology Science Coordinating Committee (BSCC), Conservation Foundation, Department of Professional Employees—AFL/CIO, Environmental Defense Fund, National Institute for Occupational Safety and Health (NIOSH), Occupational Safety and Health Administration (OSHA), Society for Industrial Microbiology, United Food and Commercial Workers International Union—AFL/CIO & CLC, and the U.S. Department of Energy. Warren C. Hyer, Jr., of the American Biotechnology Association, presided as chairman of the symposium and also served as editor of this publication.

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Introduction

Decisions on the impact upon the community of proposed new bioprocessing facilities are often made at the community level, where little bioprocessing safety experience is likely to exist. Concerned local decision makers must evaluate expert testimony and then persuade the local community (often in the face of vocal opposition) of the validity of their decisions.

The intent of the First International Symposium on Large-Scale Bioprocessing Safety, on which this publication is based, was to provide an overview of and perspective on biological safety considerations for such facilities (as opposed to electrical, mechanical, chemical, or radiological safety issues) for products that have received federal regulatory approval of their safety and efficacy. This volume also focuses on basic concepts in the safe and responsible integration of the bioprocessing facility into the local community.

The term bioprocessing is treated broadly to include traditional bioprocessing (such as the biological aspects of cheese and beer production); the production of bulk chemicals (e.g., citric acid); the production of antibiotics, vaccines, and microbial pesticides; and newer developments, such as large-scale bioprocessing using genetically modified microorganisms and mammalian cell culture techniques.

The emphasis of this volume is on the concerns of the decision maker who has no formal training in biosafety issues. This publication attempts to provide an overview of worker and community safety and health issues related to large-scale bioprocessing.

Overview of Safety in the Bioprocessing Industry

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The NIH Recombinant DNA Advisory Committee: An Example of the Self-Regulatory Process

REFERENCE: McGarrity, G. J., **"The NIH Recombinant DNA Advisory Committee: An Example of the Self-Regulatory Process,"** *Bioprocessing Safety: Worker and Community Safety and Health Considerations, ASTM STP 1051,* W. C. Hyer, Jr., Ed., American Society for Testing and Materials, Philadelphia, 1990, pp. 5–13.

ABSTRACT: The National Institutes of Health (NIH) first developed guidelines for the safe laboratory use of recombinant DNA in 1976. The NIH organized the Recombinant DNA Advisory Committee (RAC) to advise and guide work in this area. The RAC consists of biomedical researchers and members from the general public.

The NIH guidelines have been modified several times since 1976 as new data have become available. This flexibility has enabled the RAC to keep pace with the rapid developments occurring in research and development in this area. The scope, content, and methods of revision of the NIH guidelines are described. The scope and objectives of the RAC are also described. Available data suggest that the RAC has made significant contributions to the field and has created a high degree of confidence among scientists and the public.

KEY WORDS: bioprocessing, Recombinant DNA Advisory Committee (RAC), recombinant DNA, NIH Recombinant DNA Advisory Committee, molecular biology, genetic engineering

Until the 1970s, the traditional concept of laboratory safety in microbiology was that of protecting laboratory workers—and, to a lesser extent, the community—against infections by the agents being worked on in those laboratories. Subcommittees of the American Society for Microbiology have surveyed laboratory-acquired infections. The published incidences have been influenced by the agent being handled and the type of activity. Higher incidences of infection have been reported for rickettsia, arboviruses, and certain other agents. The concept of laboratory safety was greatly changed in the early 1970s, being concerned not only with the traditional aspects of infection, but also with the potential of infection from cancer and other genetic processes as a result of the procedures developed with recombinant DNA technology. Through the combined sponsorship of the National Science Foundation, the National Cancer Institute, and the American Cancer Society, the first meeting on biohazards in biological and, especially, cancer research was held at the Asilomar Conference Center, Pacific Grove, California, on 22–24 Jan. 1973. The purpose of that conference and of the resultant publication [*1*] was to consider real and potential health hazards in laboratories conducting research in animal cell biology and tumor virology.

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In the 1970s, the major documents that guided researchers in biosafety consisted of the "Minimal Standards of Biological Safety and Environmental Control" for contractors and other laboratories of the Special Virus Cancer Program of the National Cancer Institute (NCI) [2] and the "Classification of Etiologic Agents on the Basis of Hazard" [3]. In 1974, the National Academy of Sciences called for a voluntary moratorium on two types of genetic engineering [4]. This was at least in part in response to concern raised at the 1973 Gordon Research Conference on nucleic acids [5]. Such a step was unprecedented in biomedical research. Another meeting was convened at Asilomar to address this emerging technology: What was the relative risk to the laboratory worker and to the community of *in vitro* research in molecular biology? Could genes from tumor viruses or from mammalian cells cloned in bacteria be harmful to the community? Was there a cancer gene? Could cancer be infectious?

As a result of that conference, a number of academic and government researchers and specialists in biosafety began to construct suggested guidelines for the safe conduct of such research to minimize or eliminate any potential of biohazard.

Establishment of the NIH Guidelines

In the mid-1970s most of the academic biomedical research conducted in this country was supported through grants-in-aid and contracts from the National Institutes of Health (NIH). To address the concerns expressed by investigators and to contain actual and potential biohazards adequately, the NIH established guidelines on all research using recombinant DNA. The first complete document, "The NIH Guidelines," was published in 1976 in the *Federal Register* [6]. This document defined duties and responsibilities of all investigators and institutions that received NIH funding on conduct of recombinant DNA research. It also spelled out principles whereby specific applications in the field of recombinant DNA would be reviewed both at the local institutional level and at the NIH in Bethesda, Maryland. To offer counsel in the area of recombinant DNA, the then NIH director, Donald Fredericksen, established the Recombinant DNA Advisory Committee (RAC).

The RAC differed from conventional NIH committees. It included molecular biologists working in the field as well as other biomedical researchers. However, it also included members of the general public. This was a new and innovative step for the NIH; these members of the public typically were not biomedical scientists but were appointed to serve as representatives of the general public. The public members have come from such professions as law, ethics, philosophy, and public health policy and have included administrators in nonscientific fields. The public members provide valuable perspective to the RAC, offering views on issues occasionally different from those of scientific professionals. Public members have provided valuable insight to the author of this paper and to other RAC members and have frequently broadened the discussion to include views not always apparent to the professional researcher. The guidance of public members has also helped in the translation of scientific technology into risk assessment and public policy making.

The RAC also utilizes *ad hoc* consultants from various specialty areas to advise on specific issues before it. The RAC has four chartered subcommittees, dealing with these subjects: human gene therapy, plants and associated organisms, revision of the guidelines, and risk assessment. These subcommittees and other working groups offer reports, analyses, and recommendations to the RAC.

The approach to the 1976 NIH guidelines on recombinant DNA research was conservative in nature. Relatively strigent environmental containment and administrative con-

trols were placed on experiments until sufficient data were generated to show that the actual biohazardous risk was less than anticipated. If that occurred, containment could be relaxed and controls could be lessened. This approach has been the philosophy of RAC in its deliberations on risk assessment. This approach has proven prudent and effective. It has also established a high level of public confidence in the RAC on potentially sensitive issues.

Several key points of the NIH guidelines should be stressed. The guidelines are procedures which must be followed by all investigators and institutions that accept NIH funding. However, the NIH is not a regulatory agency. The NIH guidelines are just that, guidelines, not regulations. The guidelines can be amended more easily than government regulations. The method for changing the guidelines consists of publication of the proposed change in the *Federal Register* for public comment at least 30 days before the date of the RAC meeting. Typically, the RAC meets three to four times each year. Publication in the *Federal Register* requests that public comment on the issue be directed to the appropriate agency at NIH, specifically the Office of Recombinant DNA Activities (ORDA). These comments are then part of the public record for the RAC meeting. At the RAC meeting, several RAC members are assigned to review each particular agenda item, including proposed changes. The RAC will then, following discussion, usually including comments from non-RAC members, typically vote on the issue.

RAC's actions are advisory in nature. They are not themselves binding. Rather, these recommendations are forwarded to the director of the NIH for review and final action. In most cases the NIH director has followed RAC recommendations. However, there have been instances, especially when the vote was closely contested, in which the director either did not follow the RAC recommendations or sent the matter back to the RAC for further consideration.

The NIH guidelines are administered on a local level by the Institutional Biosafety Committee (IBC) of the institution receiving NIH funds. Each institution receiving NIH funding must establish an IBC, whose responsibilities need not be restricted to recombinant DNA activities. The IBC should be composed of individuals who have expertise in recombinant DNA technology and the capability to assess the safety of recombinant DNA experiments as well as any potential risk to public health or the general environment. It is the responsibility of the IBC to ensure that the NIH guidelines are followed. At least two of the members of the IBC should not be affiliated with the institution, a situation analogous to having members of the general public on the RAC.

Key components of the 1976 NIH guidelines were the definitions of physical and biological containment. Different levels of physical containment were established to contain adequately a variety of experiments whose biohazard potential ranged from none or minimal to the highest recognized levels of hazard. Initially, there were four physical containment levels, numbered P1, P2, P3, and P4. The relative biohazard risk of the experiments ranged from minimal or no risk in Level P1 through increasingly higher degrees of potential risk in Levels P2, P3, and P4. Level P4 facilities would require absolute containment, such as performing manipulations in glove boxes or requiring laboratory workers to wear ventilated space suits for protection. The workers are physically separated from the microbial cultures. The "P" notation has been replaced by biosafety levels (BL). The BL levels consider both the microbial agent being handled and the type of activity.

There is another type of containment. Biological containment consists of using organisms in experiments that are essentially crippled: that is, they either cannot propagate outside their microbial hosts or cannot propagate outside the laboratory. Two levels of biological containment exist, designated by host vector (HV) numbers, HV1 and HV2. HV1 provides a moderate level of containment. HV2 provides a higher level of containment.

In assessing the relative risk of a particular experiment involving recombinant DNA, the RAC would examine different levels of biological and physical containment. Having a higher degree of biological containment, for example, could theoretically result in the reduction of the level of physical containment to be used during experimentation.

Finally, these NIH guidelines apply to recombinant DNA research only. They do not address experiments that are outside the definition of recombinant DNA. In the NIH guidelines, recombinant DNA molecules are defined as either (1) molecules constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (2) DNA molecules that result from the replication of those described in Definition 1, just described.

Revision of the NIH Guidelines

The first major revision of the NIH guidelines occurred in 1978. The revision appeared in the 22 Dec. 1978 issue of the *Federal Register* [7]. The revision consisted of a significant simplification in the guidelines; containment and safety methods were less stringent than in the original document. This simplification and relaxation were possible only because of the accumulation of laboratory data, which documented that the risks and hazards were considerably less than originally perceived. A large number of minor revisions have occurred since 1978, effectively continuing to decrease the physical and biological containment necessary, as well as to exempt certain classes of experiments from the guidelines. Another feature of this development has been that more responsibility has been delegated to the local IBC for judgement and containment assignments. The role of RAC has changed since 1978. RAC was the first and for a long time the only governmental group that could address recombinant DNA on a scientific level of high expertise and excellence. As the technology has evolved and as it and related technologies have been applied to areas other than basic biomedical research, other federal agencies have become involved, including the U.S. Food and Drug Administration, the U.S. Department of Agriculture, and the U.S. Environmental Protection Agency. These governmental agencies have groups examining the implications and necessary regulations for manufacturing, handling, and using products containing or made from recombinant DNA. These are in line with the overall mission statements of each agency. The specific efforts of these agencies in the field of recombinant DNA is beyond the scope of this presentation. Currently, the RAC will not review applications if the subject area is regulated by another federal agency, with the exception of human gene therapy applications.

In the late 1970s through the early 1980s a number of controversial issues were presented to the RAC for consideration. One of the most controversial issues in this period was the review of applications from commercial companies rather than from academic institutions. By definition, the NIH guidelines apply only to institutions receiving NIH funds. However, commercial companies, even though they receive no NIH funding, realized that scientific overview was not available at that time in other governmental agencies. They therefore requested that the RAC review and approve plans to use recombinant DNA technology to develop and market products. This "voluntary compliance" generated controversy inside and outside the RAC. The RAC finally decided that it would review large-scale practices inherent in the recombinant DNA aspect of the application. However, the RAC reviewed the scientific nature of the application but not the facilities or bioengineering aspects of the application. Appendix K of the NIH guidelines addresses the large-scale aspects of recombinant DNA. By definition, the term large-scale refers to applications of more than 10 L of volume of the recombinant DNA culture.

The Present NIH Guidelines

The NIH guidelines for research involving recombinant DNA molecules are described in the *Federal Register* [8]. The NIH guidelines in effect in 1987–1988 are listed in the 7 May 1986 issue. These are updated periodically to reflect major actions by the RAC that have been accepted by the director of NIH. The applicability of the guidelines, the definition of recombinant DNA, and the roles of the RAC and the IBC are essentially the same as those described in the 1976 guidelines. Table 1 lists the general contents of the NIH guidelines.

Essentially four types of experiments are defined by the guidelines:

(*a*) experiments that require RAC review and NIH and IBC approval before initiation,
(*b*) experiments that require IBC approval before initiation,
(*c*) experiments that require IBC notification at or before initiation, and
(*d*) exempt experiments.

Experiments That Require RAC Review and NIH and IBC Approval Before Initiation

There are four different types of experiments in this classification. All these experiments, in the opinion of RAC, require thorough scientific review before they can be implemented. The potential risks are higher in this group than in the other groups of experiments covered. Thorough case-by-case review is crucial for these proposed experiments.

1. Deliberate Formation of Vertebrate Toxins—The first group of experiments in this classification involves deliberate formation of recombinant DNA containing genes for the biosynthesis of toxic molecules that are lethal for vertebrate animals at a median lethal dose (LD_{50}) of less than 100 ng per kilogram of body weight. Examples include botulism, tetanus, and diphtheria toxins. For work on these and related toxins, investigators are

TABLE 1—*Major components of the NIH guidelines on recombinant DNA.*

Component	Area Covered
Section	
I	scope of the guidelines
II	containment
III	guidelines for covered experiments
IV	roles and responsibilities
V	footnotes
VI	voluntary compliance
Appendix	
A	exempted experiments
B	classification of microorganisms according to hazard
C	other exempted experiments
D	actions taken under the guidelines
E	certified host vector systems
F	conditions for cloning toxin genes
G	physical containment
H	shipment of agents
I	biological containment
J	biotechnology science coordinating committee
K	large-scale applications
L	release into the environment of certain plants

referred to Appendix F of the NIH guidelines, which more thoroughly describes the type of containment and procedures necessary to construct such toxin molecules. ORDA has on file a list of toxic molecules that are classified according to their LD_{50}s. These were prepared from a variety of sources, including the RAC Working Group on Toxins.

2. Deliberate Release into the Environment of Any Organism Containing Recombinant DNA—Deliberate environmental release represents a special situation. The original purpose of the NIH guidelines was to provide the proper level of laboratory containment. Environmental release, on the other hand, deliberately breaks containment and allows the recombinant organism access to the general environment. Examples include recombinant microorganisms to prevent frost damage, genes that have been incorporated to confer pesticide resistance, and bacterial and viral vaccines developed with recombinant DNA techniques to be administered to animals or humans. In this category, to date, NIH permission has been granted to field-test corn, tomato, and tobacco plants modified by recombinant DNA under specified containment conditions. Permission has also been granted to release, under specified conditions, *Pseudomonas syringae* which are devoid of genes that are involved in ice nucleation. These bacteria have been referred to in the popular press as ice-minus bacteria.

Deliberate release experiments can be performed with certain plants containing recombinant DNA, as described in Appendix L of the NIH guidelines, if approved by the Plant Working Group of the RAC. Investigators wishing to conduct such experiments must submit an application to NIH for review by the RAC Plant Working Group and for specific approval by NIH. Such experiments also require IBC approval before initiation. The major criteria for allowing such a release are that the plant species involved must have no relative known to be a noxious weed and that the introduced DNA and vector must consist of well-characterized genes and sequences. Plants must be grown in a controlled-access field under specified conditions appropriate for the plant under study and the geographical location. These criteria were established by the RAC Working Group on Release into the Environment.

This same working group on environmental release also developed a list of Points to Consider for submissions to the RAC involving testing in the environment of microorganisms derived by recombinant DNA techniques. This appendix is not part of the NIH guidelines but is available from ORDA. It has been published in the *Federal Register,* as well as in a text entitled *Biotechnology Risk Assessment* [*9*]. Other appendices for the guidelines are in the planning stage for environmental release of animals and vaccines containing recombinant DNA to the environment.

3. Deliberate Transfer of a Drug-Resistant Trait to Microorganisms That Are Not Known to Acquire It Naturally—The concern of RAC in this area is that such practices, if performed frequently and without supervision, could significantly increase the level of drug resistance in human pathogens. Acquisition of antibiotic resistance could result in compromising the usefulness of the drug for controlling disease agents in human or veterinary medicine or agriculture.

4. Deliberate Transfer of Recombinant DNA or DNA or RNA Derived from Recombinant DNA into Human Subjects—The Human Gene Therapy Subcommittee of RAC has recently submitted a preclinical data document on potential use of recombinant DNA in human gene therapy [*10*]. This document does not represent a clinical proposal, and to date no clinical protocol for actual gene therapy has been submitted to the RAC for approval. A proposal for gene transfer into human cells has recently been approved. The objective of this preclinical document is to present background information and definition of certain questions that should be addressed in the approval of any human gene therapy

protocol. In this area, the RAC and its subcommittee clearly distinguish between potential gene therapy performed on somatic cells and therapy performed on sex cells, since such genetic manipulations on sex cells would not only result in the "curing" of a trait but also would present an additional genetic burden to succeeding generations.

Experiments Requiring IBC Approval Before Initiation

At the present time, probably most of the recombinant DNA experiments performed in academic biomedical research are in this category. The information requested by the IBC would generally be about the source of the DNA used in these experiments, the nature of the DNA (what genes or sequences) that will be inserted into a recipient host, the host and vectors to be used, and whether a deliberate attempt will be made to obtain expression of a foreign gene and, if so, what protein will be produced. The IBC will also request from the submitter any information regarding containment conditions for this type of experiment that are specified in the guidelines. Specific guidance regarding containment levels can be obtained by reference to the NIH guidelines on infectious agents themselves [*8*] as well as to the monograph "Biosafety in Microbiological and Biomedical Laboratories," published by the Center for Disease Control and the NIH [*11*]. Investigators and IBCs are urged to refer to the NIH's "Laboratory Safety Monograph" [*12*] for more specific information regarding containment facilities.

Experiments That Require IBC Notification at or Before Initiation of Experiments

Experiments that are not clearly defined in the previous sections can be carried out at a low biosafety level (BL1) containment. The investigator must simply sign and date a registration document and file it with the IBC. The IBC still reviews these experiments and maintains a listing of all recombinant DNA experiments that are ongoing at the institution. However, IBC approval prior to initiation is not necessary.

Exempt Experiments

During the past eleven years, certain categories of experiments have been defined as exempt from the NIH guidelines because either they pose no biohazard or they do not fall under the definition of recombinant DNA. Such experiments include those which are not in organisms or viruses, that is, those performed only with synthetic chemicals. Also exempt are experiments that consist entirely of DNA segments from a single nonchromosomal or viral DNA source. The NIH has exempted those molecules that consist entirely of DNA from a prokaryotic host, including its indigenous plasmids or viruses when propagated only in that host, or in a closely related strain of the same species, or when transferred to another host by a well-established physiological means. This would include recombinants that consist entirely of DNA from a eukaryotic host, including its chloroplasts, mitochondria, and plasmids. The RAC has also exempted specified recombinant DNA molecules that consist entirely of DNA segments from different species that are known and demonstrated to exchange DNA naturally by known physiological means. A list of such natural exchangers is listed as Appendix A to the guidelines.

Scope of Present Activities and Future Directions

As stated, one of the key features of the NIH guidelines is that they are that, guidelines and not regulations. As such, they are flexible and can be more readily amended as a result