

United States

Office of the Administrator

SAB-EETFC-88-010

Environmental Protection

Science Advisory Board

January, 1988

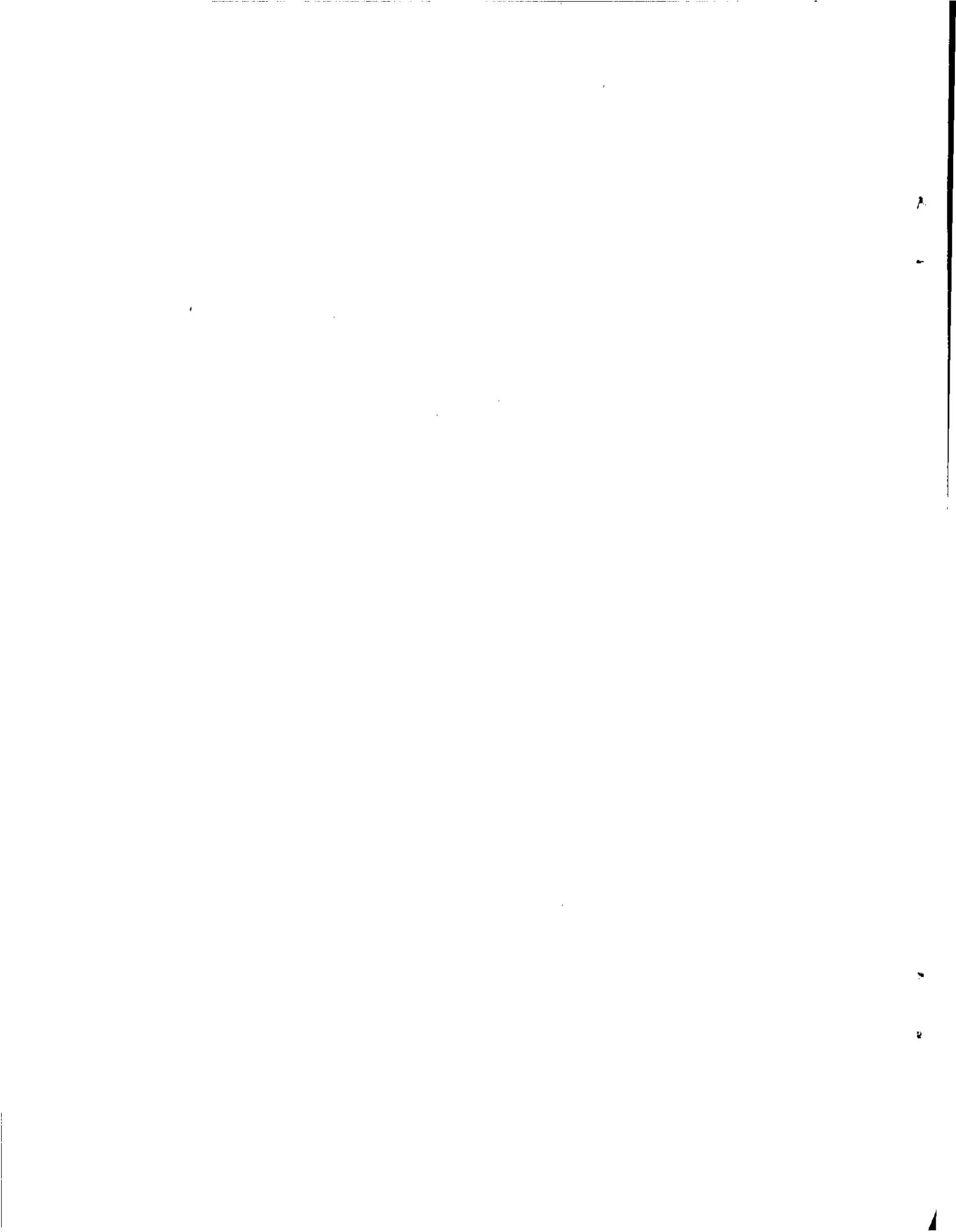
Agency

Washington, D. C. 20460



Report of the Biotechnology Research Review Subcommittee

Evaluating EPA's Current Objectives and Future Needs for Biotechnology Risk Assessment Research





UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

January 29, 1988

OFFICE OF
THE ADMINISTRATOR

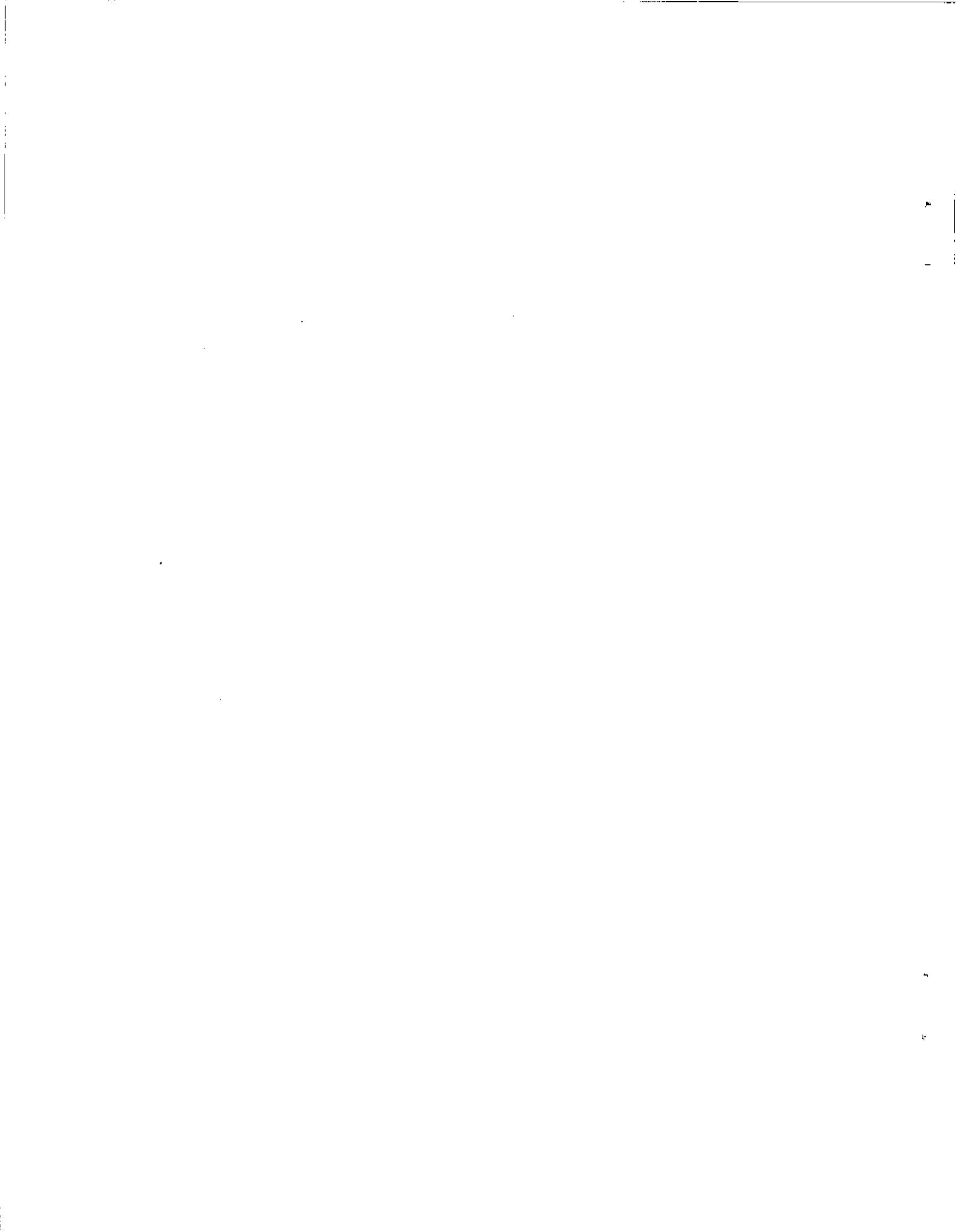
The Honorable Lee M. Thomas
Administrator
U.S. Environmental Protection Agency
401 M. Street, S.W.
Washington, D.C. 20460

Dear Mr. Thomas:

The Biotechnology Research Review Subcommittee of the Science Advisory Board's Executive Committee has completed its review of the Office of Research and Development's (ORD's) Biotechnology/MPCA Risk Assessment Research Program. The review was requested by the Assistant Administrator for ORD, and was conducted on June 22 and 23, 1987 in San Diego, California.

The Subcommittee recognizes the significance of the accomplishments achieved by the program to date, primarily in developing methods for enumeration, identification and detection of genetically engineered microorganisms (GEMS) as a foundation for monitoring strategies. In light of this progress, a shift in focus is recommended towards: 1) determining impacts of GEMS on environmental processes and assessing the potential for producing ecological effects; 2) developing control strategies based on built-in biological mechanisms for promoting containment and mitigation; and 3) applying increased knowledge to development and refinement of protocols, microcosm studies and predictive models.

Increased emphasis is also recommended on investigations into gene expression. The physiological basis for exchange of genes should be investigated. Research is also recommended to determine the potential for environmental change that may result as genes from introduced GEMS are transferred to naturally occurring organisms or as organisms at specific sites transfer into released GEMS.



The Subcommittee has concerns with the limited scope of the health effects component of the research program and believes that this research has not been framed around the principal health-related problems and issues that may arise. The Subcommittee supports the engineering research component, and recognizes the economic use of existing information at its foundation. Some concern was expressed over the time table for implementation of the research program components, since some of the tools likely to be developed may be needed before the schedule allows such development.

Additional recommendations are offered concerning training, proposal solicitation, and cross-communication. Modifications are suggested to the system for soliciting extramural research projects, and the creation of a variety of grant and contract types is recommended. Although communication with the academic sector is considered strong, research ties need to be strengthened with the private sector, where relevant research is being conducted. Finally, communication within the Agency should be strengthened, particularly between the staff of the Athens Environmental Research Laboratory and the staff developing the Biosystems Initiative.

The Subcommittee appreciates the opportunity to conduct this scientific review. We request that the Agency formally respond to the scientific advice transmitted in the attached report.

Sincerely,



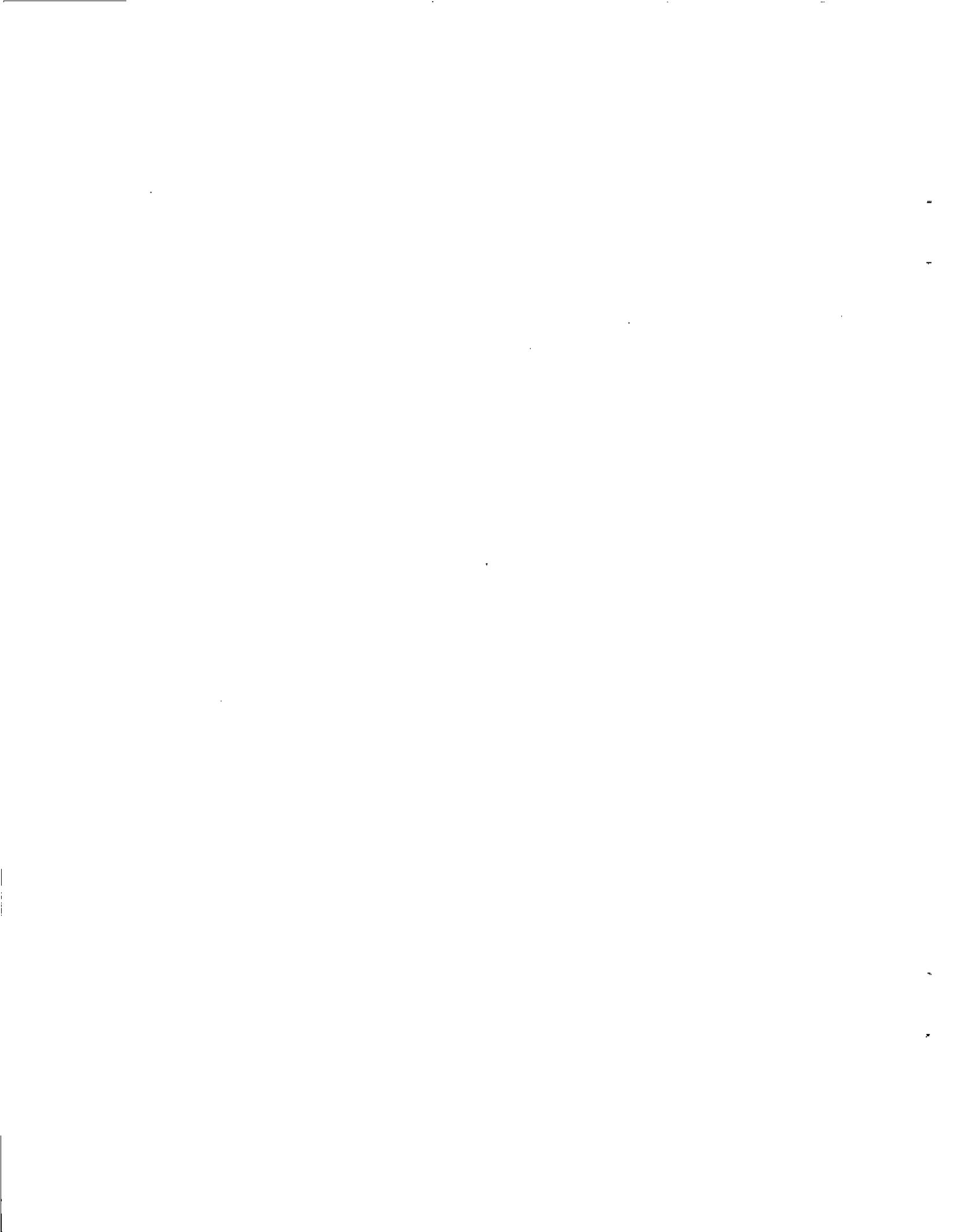
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REPORT OF THE BIOTECHNOLOGY RESEARCH REVIEW
SUBCOMMITTEE

Evaluating EPA's Current Objectives and
Future Needs for Biotechnology
Risk Assessment
Research

U.S. Environmental Protection Agency
Science Advisory Board
Washington, D.C.

January 1988

U.S. ENVIRONMENTAL PROTECTION AGENCY

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1.0 EXECUTIVE SUMMARY

In general, the Subcommittee concludes that the research program has produced significant accomplishments in a number of areas in a short period of time. Preliminary areas of concentration on the development of methods for enumeration, identification and detection of organisms with both selective media and nucleic acid probes were appropriate, and provide an acceptable foundation for monitoring strategies. While promising methods should continue to be developed, the Subcommittee believes that the research program is now at the point where emphasis should be shifted to apply the methods that have been developed. Therefore, the major recommendation of the Subcommittee is that EPA redefine its focus to emphasize three areas: 1) investigating and analyzing environmental effects, 2) developing control strategies for containment and mitigation, and 3) refining further applications such as protocols, microcosms, models and field tests.

Environmental and ecosystem effects studies should identify specific ecological processes, such as nitrification or sulfur reduction, and solicit or develop projects to study the impact and interaction of genetically engineered microorganisms (GEMs) on these target processes. Analysis of past environmental perturbations and species displacements resulting from chemical amendments may be useful for targeting endpoints for investigation, protocol development and establishment of positive controls. A long-term component should be added to ecological effects research to examine the potential for chronic and/or cascading effects. The data generated should be used in tandem with existing data, such as those developed by EPA's Environmental Research Laboratory at Athens, Georgia. In short, a greater effort should be made to capture and incorporate existing, pertinent data to strengthen modeling and effects assessment efforts.

The Subcommittee supports the innovative approach taken at EPA's Environmental Research Laboratory at Gulf Breeze, FL, to develop control mechanisms, such as suicide plasmids. This approach to containment and mitigation was considered to be especially important for EPA to pursue, because such strategies reduce the risk associated with environmental release of these organisms. Chemical and physical methods for control should be investigated along with biological methods to reduce the need for remediation and facilitate emergency response procedures. Investigations should assess the environmental consequences that may be posed by control methods, as well as the degree of control achievable.

The Subcommittee recommends that developments made in the identification and detection area be incorporated into protocols for assessing and predicting the effects and/or risks posed by the products of biotechnology. These applications are essential for EPA's program offices as they examine and make decisions about new products. The development of microcosm protocols

should continue with increased emphasis on standardization, validation, calibration and quantification of environmental effects. Increasing complexity and predictive capability should be built into models, by capitalizing on existing Agency experience in other areas and by validating to insure that reasonably accurate predictions are produced. A sufficient number of models should be developed to encourage predictions that are relevant to the effect of concern, rather than relying on a single or a few models to predict likely effects in all situations. The Agency should also begin to plan for actual field studies with engineered organisms to validate applications and develop new knowledge.

In addition to whole cells, control of genes and mobile genetic elements should be investigated. This investigation should include research into the physiology of gene exchange, and the effects of genetic changes on individual cells, populations, communities, and, ultimately, the ecosystem.

The Subcommittee has concerns with the limited scope of the health effects research component, especially in light of the importance of this line of research. Some members question the soundness of EPA's decision to conduct research on health effects, especially since the underlying strategy for health effects research has not been framed around the health related problems and issues that may arise. Funding for the program has been minimal and cutbacks have been experienced for two succeeding years, making EPA's commitment to this program a questionable matter. This component is the weakest part of the program and leaves the Agency open to criticism. Considerable effort should be expended to develop a strategy that addresses likely routes of exposure and potential for effects, while supporting both Subdivision M guidelines and EPA mandate.

Mechanisms for implementing this strategy should include extramural research grants to institutions with appropriate expertise for conducting health effects investigations. Alternatively, EPA's emphasis should be placed on detecting exposure and on developing model systems while expanded health effects research on relevant organisms becomes the focus of other agencies. For example, certain health effects problems may be more appropriate to the mandate and expertise of agencies such as the Centers for Disease Control (CDC) or the, or the National Institute for Occupational Safety and Health (NIOSH). Since a previous SAB Study Group on Biotechnology also recommended that EPA sharpen its health research component to focus on information needs for risk assessment and policy analysis, the Subcommittee strongly reiterates that EPA has not developed a scientifically relevant health research program to serve the Agency's needs.

The Subcommittee supports the engineering research component, and recognizes the interweaving of extensive existing information and intra-agency communication at its foundation. The engineering component of the program is in a very early stage, and the time table for implementation extends far into the

future. The Subcommittee has concerns that some of the tools and applications likely to emerge from this research will be needed before the schedule allows their development.

The Subcommittee made two additional suggestions that may enhance the research program: 1) develop experimental designs that take advantage of the molecular uniqueness offered by the DNA, RNA and protein structures of GEMS as compared to native microbes and 2) consider the potential for debilitating organisms to enhance control and containment.

Finally, the Subcommittee offers recommendations concerning training, proposal solicitation, and cross communication. Biotechnology is an area where EPA has the opportunity to recruit and retain highly qualified graduates, and small research grants or fellowships offered to students could pave the way while strengthening interaction between academia and EPA. This approach should be examined to help provide a well trained, committed staff in genetic engineering at EPA.

The Subcommittee recommends some modification to the system of soliciting extramural research programs, and the creation of a variety of grant or contract types to better meet specific needs. Cross-communication with the academic sector of the scientific community is considered appropriate; however, stronger research ties should be established with the private sector. This communication is important, since a large body of relevant research is funded and conducted by industry and duplication of effort should be avoided. Cross-communication can be enhanced within the Agency by capitalizing on information that has been generated through modeling projects at EPA's Environmental Research Laboratory at Athens, Georgia, and information that may be generated in the future by such programs as the Biosystems Initiative. Because of the scope of the program, and the relationship between biosystems for pollutant degradation and Microbial Pest Control Agents (MPCAs) in terms of environmental effects, the Subcommittee recommends that the Biosystems Initiative undergo independent peer review prior to final program design and implementation.

2.0 INTRODUCTION

2.1 Request for Science Advisory Board (SAB) Review

At the request of the Assistant Administrator for the Office of Research and Development (ORD), the Science Advisory Board's Executive Committee agreed to carry out a scientific review of the EPA's Biotechnology Risk Assessment Research Program. The Executive Committee authorized the formation of a Subcommittee to perform this review, which focuses on biotechnology risk assessment/microbial pest control agent (MPCA) research carried out to support the Office of Pesticides and Toxic Substances.

This action is part of a continuing series of research program reviews that have been conducted by the SAB. The purpose of these research reviews is two fold. First, the reviews are designed to provide independent scientific advice on the objectives, quality and relevance of ongoing research, particularly with regard to meeting the Agency's regulatory requirements. Second, the research reviews evaluate the content and direction of the programs as they support and anticipate future Agency needs.

In addition to research program reviews, the SAB has also participated in other activities related to biotechnology. In 1985, the SAB convened a Study Group on Biotechnology. This group issued a final report, Assessing EPA's Biotechnology Research and Information Needs, in January of 1986. A summary of the Study Group's findings is provided in section 2.3.1 of this report. The SAB also consults and coordinates with the Agency's Biotechnology Science Advisory Committee, assessing issues requiring research and referral to appropriate EPA research committees and Program Office Directors.

2.2 Subcommittee Review Procedures

The Biotechnology Research Review Subcommittee met on June 22-23, 1987, in San Diego, California. Briefings were provided on the historical perspectives and environmental research components of the Biotechnology/MPCA Risk Assessment Program by ORD staff. Additional briefings highlighted the objectives of ongoing biotechnology research projects within the Office of Environmental Processes and Effects Research, the Office of Environmental Engineering and Technology Development, and the Office of Health Research. Future research plans were also presented by these three Offices. A document entitled "Status Report for Biotechnology/Microbial Pest Control Agent Risk Assessment Program" was submitted to the Subcommittee prior to the meeting to provide more detail on the program framework and the intramural and extramural research projects that comprise the program (see Appendix II). These projects are being conducted by scientific staff at the Environmental Research Laboratories in Gulf Breeze, Florida; Corvallis, Oregon; Duluth, Minnesota; and Research Triangle Park, North Carolina, and by collaborating academic and industrial laboratories.

Representatives from regulatory program offices including the Office of Pesticide Programs (OPP) and the Office of Toxic Substances (OTS), defined the ways that this research is used to support regulatory decision making and identified particular research needs related to their respective regulatory requirements. These regulatory needs from OPP's perspective primarily include support and development of testing guidelines. Needs for short-term tests, environmental identification procedures, and subchronic and chronic testing protocols were identified, as well as intratracheal exposure methods development and test design.

To meet OTS goals, risk assessment methods need to be developed and expanded. In particular, methods for detecting and predicting ecological effects are required, including cascading effects on ecosystems. Validation of current test methods and applications, such as systems modeling, are also needed for enhancing risk assessment abilities within OTS.

2.3 Results of Prior Peer Review

2.3.1 SAB Study Group on Biotechnology

In January of 1986, the SAB completed a preliminary examination of EPA's research and risk assessment capabilities associated with the field application of genetically engineered organisms through its Study Group on Biotechnology. This Study Group was charged with a) identifying information gaps for performing risk assessments on the products of biotechnology, b) exploring the direction of EPA's research program in biotechnology and c) evaluating the availability of testing procedures and assessment methods for genetically altered organisms.

The Study Group found that work had already been initiated by the Agency in several areas where critical research needs exist, including survival, growth, and genetic transfer. However, it also identified areas that need additional attention such as, dispersal, remedial action, and environmental effects. In addition, the planning for health effects research was considered to be limited in scope and in need of expansion and additional resources.

The Study Group recommended that EPA give a higher priority to developing test protocols for compliance with policy and regulatory requirements, continuation of efforts to promote interagency and international communication and cooperation, and establishment of an advisory panel to review research, testing procedures and risk assessment approaches on a continuing basis.

2.3.2 Panel for Peer Review of the Biotechnology/MPCA Risk Assessment Research Program

On May 4-8, 1987, the Office of Research and Development convened a Peer Review Panel at EPA's Environmental Research Laboratory in Gulf Breeze, Florida. This panel was charged with evaluating the scientific quality of individual research projects that are conducted under the Agency's Biotechnology/MPCA Risk Assessment Research Program, and with making recommendations for program improvements. Individual experimenters, both intramural and extramural, presented their research findings and status to facilitate review of program areas. These areas include survival, stability, and gene transfer of genetically engineered microorganisms in microbial communities, ecological effects of such organisms in both environmental samples and microcosms, and assessment of risks posed by microbial pest control agents.

The panel cited several meaningful program accomplishments. For example, the program has demonstrated the promise of nucleic acid probes as tools for tracing novel gene sequences. Several projects related to microbial ecology were of special interest. Three innovative methods for recovery by plating were recognized as technically efficient and cost effective. The development of approaches using conditionally debilitated organisms to enhance the capability to control environmentally released organisms was applauded as an effort; however, considerable refinement must occur before this concept can be applied. Finally, the interactive use of computer models and extrapolation from microcosm to the field were considered appropriate.

The Peer Review Committee's recommendations include expanding the diversity of organisms for investigation. The program now focuses on organisms likely to be used for genetic engineering, rather than on familiar, genetically malleable organisms that are environmentally relevant. Recommended improvements include the use of a broader spectrum of organisms including gram positive bacteria, spore formers, anaerobes, cyanobacteria, slow growing, and oligotrophic bacteria. Environmental isolates should be added to the research program, in addition to the currently used laboratory cultivars.

The continued reliance on antibiotic resistance markers for survival and gene transfer studies is encouraged; however, there are problems with this approach. The development of other methods to eliminate the potential for the spread of these genes in nature is necessary. Concern can be reduced by incorporating antibiotic resistance markers onto the chromosome, as opposed to locating them on less stable plasmids.

Problems have arisen in determining survival of engineered bacteria. The greatest problems in determining survival seem to stem from presumptions that the phenotypes expressed by cultures of microorganisms grown under laboratory conditions will not change when the microorganisms are introduced into environmental settings. Researchers also presume that the expressions of

phenotypic traits will be immediate when microorganisms are recovered from the environment and are grown under laboratory conditions. These presumptions are not proving to be dependable; therefore, research efforts to enhance recovery by physiologic support rather than imposed selection should be pursued.

The survival of introduced organisms is usually compromised, probably because of their poor ability to compete with naturally occurring biota. Physiologic support may improve the ability of the organisms to survive and compete if such support focuses on the physiologic conditions of the microorganisms at the time of environmental release. In the past, microorganisms intended for release at natural sites have been prepared under artificial conditions that encourage their rapid growth, rather than preparing them for the environmental conditions that they will meet upon release. Means of pre-conditioning microorganisms prior to release should be investigated to improve their survival and competition in natural sites. Pre-conditioning approaches may include nutrient-restricted cultivation, adsorption onto particles or culturing at ambient temperatures. Research into pre-conditioning microorganisms should also encompass means for enhancing and ensuring the expression of genetically endowed phenotypes under ambient environmental conditions.

The panel recommended that studies of dispersal be added to the research program, especially in the aquatic environment. Dispersal studies should be designed to aid in validating microcosms and modeling efforts and to enhance monitoring strategies. Finally, since the ultimate testing must be conducted in the outdoor environment, the Agency should design and conduct experimental protocols in the field.

This panel, convened for review of the Biotechnology MPCA Risk Assessment Research Program, was charged with reviewing the work of the individual researchers and the merit of the projects conducted by them in relation to the objectives originally defined for the program. In contrast, the Science Advisory Board Biotechnology Research Review Subcommittee focused solely on oversight of this research program, evaluating the objectives and future directions of the program in light of Agency needs. Since some similar issues were examined by each, some overlap in membership between the two committees facilitated a coordinated approach.

3.0 SUBCOMMITTEE EVALUATION OF EPA'S BIOTECHNOLOGY RISK ASSESSMENT RESEARCH PROGRAM

Considering that this program was initiated in 1985, with extramural projects funded for the first time in 1986, considerable progress has been made. Initial areas of concentration on enumeration, detection and identification of organisms have provided a useful foundation. Experiments with selective media allow detection of culturable organisms, while the application of gene probes enables detection of specific gene sequences. The use of these techniques provides the ability to monitor both organisms and genes, and the Subcommittee considers the monitoring portion of the program to be both scientifically acceptable and relevant to EPA's needs.

The Subcommittee recognizes that the research program supports regulatory decision making, which occurs under the pressure of time. Decisions concerning the environmental release of organisms that can be exceedingly diverse in ecological function must often be made 90 days after information is received from those seeking permission to conduct field testing. This factor and other requirements of a regulatory risk assessment process were considered as the Subcommittee conducted its review.

3.1 Methods Development - Survival, Stability and Gene Transfer in Microbial Communities

Satisfying data needs for tracking and assessing microorganisms, both quantitatively and qualitatively in environmental communities, depends on the ability to develop effective methods to provide such data. Thus, it is not surprising to see an early emphasis in this research program on methods development.

The Subcommittee evaluated the general objectives of methods development in relation to genetically engineered organisms and microbial pest control agents as a part of the review. Selective media methods still remain the single cheapest, simplest method for detecting microorganisms. However, new technologies can be derived from selective media approaches, and these should be aggressively pursued. Detection of DNA sequences and their gene products with more "fashionable" methods, such as gene probes, has not proven to be as practical or as sensitive as improved classical methods.

Advances in microcosm technology were also achieved by research conducted under this program. Microcosm systems were established and incorporate sampling techniques for detecting both microorganisms and DNA. The microcosms described were used to study environmental effects, primarily biodegradation, and for survival studies on epiphytic bacteria that have been modified. Additionally, gene exchange frequencies were studied using microcosms.

An entirely different approach, one of containing microorganisms rather than monitoring them, was included in the research projects being conducted at EPA's Gulf Breeze Laboratory. This experimental approach involves the development of "suicide" plasmids, which cause the death of the microbial cell under conditions determined by the investigator, such as after a pollutant is degraded. These advances reduce the possibility of plasmid genes escaping beyond the release site, and may even reduce horizontal transfer rates. The particular suicide systems under development may have characteristics that are less than ideal, but this approach represents a highly innovative and serious step to enhancing containment.

The Subcommittee considers the advances in containment methods to be especially important for EPA to pursue, since they directly support the Agency's mandate of protecting human health and the environment. Other types of methods research can and are being conducted by microbiologists in industry and academia, but EPA should play a major role in containment research. Development of such genetic and biologic controls could reduce the potential for unforeseen consequence associated with releases of both microorganisms and novel genes.

3.2 Environmental Analysis - Survival, Stability and Gene Transfer in Microbial Communities

While significant progress has been made in methods development, the primary goal for the current time frame (1-2 years) should be shifted toward environmental analysis. This shift should emphasize using the methods developed and testing their utility in experimental environments and the field. Over time, this direction should become a major program focus. At present, some projects with methods development objectives could be reoriented to reflect this shift in direction.

Analysis of gene transfer in the environment is a crucial part of the research program. Of particular importance is the role played by conjugation, or genetic transfer from donor to recipient via direct cell contact. Experience with the dissemination of drug resistance genes and plasmids in nature clearly supports this contention. However, the molecular and physiological events accompanying such conjugal transfer are not understood, except in a very few, well characterized systems. This lack of understanding complicates any approach by increasing the number of variables in an already ill-defined system. Physiologic state, metabolic requirements for transfer, cell density, need for intimate cell contact, and the role of competing cell populations are examples of such variables. These issues should be addressed as studies are expanded to include more varieties of taxonomic groups as experimental candidates.

Where practical, special attention should be directed toward investigating the fate of the mobile genetic elements carried by genetically engineered microorganisms. Plasmids, insertion

sequence elements, and transposons afford genetic plasticity that could have striking effects on both genotype and phenotype. This may be magnified by mobility, both acquisition and loss, of such elements at the intergeneric level. Detection and environmental analysis should be emphasized for monitoring the dynamics of the mobile gene pool. The fact that plasmids with potential utility for tracking engineered microbes in nature might be rapidly lost is offset by the fact that genes of interest may be retained within more stable plasmids or chromosomes.

3.3 Ecosystem Effects

The overall goal of the ecosystem effects portion of the Agency's research program is to assess the impact of the release of GEMs on processes and populations found in natural habitats. While similar, the goal for MPCAs is the identification, design, protocol development, and validation of testing methods needed by a registrant approaching EPA with a request for MPCA release.

Research issues related to ecosystem effects which remain to be addressed by the Agency include:

1. Determination of the effects of GEMs on various components of ecosystem structure and function. This includes non-target organisms (individual as well as species and communities), mineral cycle processes, and the effects of plants as components.
2. Identification of geochemical and biological processes likely to be most useful as indicators of GEM effects.
3. Development of model systems for predicting impact, alterations, and recovery of populations, communities, and ecosystems exposed to GEMs.
4. Field studies to verify model systems, microcosm studies, biological indicators, and ecosystem responses to released GEMs.

The ecosystem effects portion of the current EPA biotechnology research program is in its initial stages and most research efforts have appropriately centered on the development of protocols, GEMs, detection probes and methodologies necessary for examining the issues 1-4 above. The current research is reasonably diverse in the habitats that are being explored, effects on non-target animals, and ecosystem studies, although it is limited with respect to the bacterial (and fungal) organisms chosen for probe development.

Some progress is also being made in microcosm studies of varying complexity and on modeling systems that will be useful for examining effects of released GEMs. The modeling approach has been to develop a single, complex model of microorganism

growth and dispersal. Greater pluralism in model development should be encouraged. Hopefully, a pluralistic approach will result in a number of models that can be used to assess different situations, and answer different types of questions, including the functional role of the microorganisms in the ecosystem, the type of environment receiving the organisms, and the dispersal or growth strategy employed by the microorganisms.

3.4 Health Effects

Current health effects research projects are conducted at two branches within EPA's Health Effects Research Laboratory in Research Triangle Park, NC. One branch conducts research involving microbial pest control agents and consists entirely of intramural research efforts. Although no extramural grants are included in this portion of the program, viral research efforts are supported by Northrup Services, Inc., an on-site contractor. A second branch, the Genetic Bioassay Branch, primarily investigates the colonization of the rat gut by Pseudomonas aeruginosa and P. maltophilia.

The underlying objectives used to develop the health component of the research program were not clear to the Subcommittee. The overall program suffers from too little coordination of effort between the two branches; for example, MPCAs are not used as test organisms in the Genetic Bioassay Branch. Too little manpower is provided to make real advances in this important area. The program also fails to ask the proper questions relating to human health issues. The first question to be asked is whether the route of concern is work-place exposure or incidental exposure to the general population. This will determine the route(s) of exposure to be used in the research, and the experimental questions and designs should follow accordingly.

If the program's objectives are to evaluate work-place exposure, the primary route is certain to be inhalation, though exposure through other routes is possible. The research directed toward developing a validated alternative to inhalation chambers is a necessary first step and one which meets the presumed program objectives. Mammalian cell culture testing, where selected tissues (epithelial, nasal, or pulmonary) are tested to determine changes in infectivity and or pathogenicity resulting from the engineered organism, is also appropriate. It makes sense to do this with MPCAs that are produced and disseminated at this time. Appropriate adjuncts to the tissue culture work are studies on the immune responses to engineered microorganisms. There are two immediate questions to be answered: 1) do GEMS elicit a meaningful immune response, and if so, what is the nature of that response and, 2) is the response different from parental, non-genetically engineered organisms? These investigations will require whole animal studies as well as studies involving colonization/clearance of target organs.

If, on the other hand, the program's objectives are to evaluate the general public health hazard of incidental exposures to GEMS, then the answers to the first question are quite different. The most likely routes of exposure are ingestion of materials contaminated by GEMS or exposure of wounds to contaminated matter. In these two scenarios the studies should evaluate different situations. In any case, all studies will have to be made with relevant GEM organisms and wild-type parental strains. Again, MPCAs would seem to be the logical starting place for such studies. In some cases, a few well designed experiments will yield important insights, while, in other cases, appropriate data will have been submitted as part of the application package. In yet other cases, extensive, relevant research will be needed.

3.5 Engineering Research

Since the engineering research program is in the very early stages, little evaluation of ongoing research was possible. Subcommittee recommendations have more relevance to future needs of the program and can be found in the following section.

4.0 SUBCOMMITTEE RECOMMENDATIONS

4.1 Future Needs of the Biotechnology Risk Assessment Research Program

4.1.1 Methodology Needs

When a new technique is in vogue, its application may become over-extended to problems which could easily be solved by procedures already in use. Use of the most advanced techniques may be perceived as the strongest aspect of a research proposal even though they are not the best, the most sensitive, or the most appropriate. This factor should be kept in mind by the investigators and the managers of the research program.

The program's emphasis to date on methods development should probably now shift from detection systems to self containment systems. One such system, the suicide cassette, has been proposed, and there should be a call for proposals to develop similar systems in diverse groups of bacteria and fungi.

Containment and mitigation measures can be developed by incorporating chemical, physical, and biological approaches. Biological containment possibilities can be developed by investigating taxonomic restrictions as they interact between GEMs and their targets, such as plants, free living microorganisms and pathogenic microorganisms. Metabolite requiring systems may offer possibilities for control. Controls may be imposed on genes, or on expression of the genes. Insertion of new sequences and traits directly into the chromosome, rather than on less stable plasmids, enhances the potential for following the gene. Development of such risk reduction methods could greatly affect the expanding responsibility for EPA to review and regulate microbial releases, and would serve to streamline the requirements for conduct of tests as well as application and review processes.

Since considerable progress was made from 1985 to the present with regard to basic developments in probe research, the research program now needs to move on to the practical application of these techniques, especially in the field. Development of data to assess ecological effects and risk should begin with microcosm studies. Data derived should be used to provide a catalog of information on detection methodologies, specific organisms, and selected groups of organisms.

Future efforts need to distinguish between planned releases, where organisms are investigated and characterized prior to their environmental application, and inadvertent releases where the organisms are not designed to be detected in the environment. Organisms designed to be released have marker traits to facilitate environmental monitoring or mitigating traits to control their establishment and dissemination. Organisms commonly used in fermentation or other industrial, contained systems may not have such traits. Therefore, inadvertent

releases could be a problem, especially since the methods already developed may not be applicable. Further research avenues should be pursued to investigate this potential problem.

4.1.2 Environmental Analysis Needs

Research has progressed fruitfully in identifying and tracking GEMs. The information gained from this research should now be organized, retained and made available by establishing a database of microbial strains, GEMs, vectors, plasmids, and probes to facilitate ecological studies. In addition, a collection of the methods used and validated for specific purposes should be collated as a reference for researchers. Both tools should be expanded as new information becomes available, especially as tracking of GEMs is expanded to microcosm testing and actual field situations.

Some progress has also been made in establishing control over organisms after their deliberate release. Secondary intervention, where the environment is amended with chemicals that the organism is sensitive to, is often used and may be appropriate. However, the environmental consequences of these methods should be investigated. The most appropriate control methods appear to be biological, where host ranges are narrowly restricted, or when organisms are genetically constructed so that their survival is no longer possible when the agent they were created to destroy is no longer present in the environment. The investigation of additional, built-in biological mechanisms of control should be encouraged. Mechanisms for control of genetic material, rather than organism viability, should also be investigated, since elimination of the organism may not result in eradication of introduced genetic traits. A greater understanding of mobile genetic elements and gene sequences themselves is necessary to predict the potential for gene incorporation and/or expression in organisms that inhabit environments where releases are to occur.

The program to date has placed some emphasis on the potential for gene exchange. New efforts should build on these, focusing on both the physiological basis and the evolutionary consequences of the genetic change produced. Fundamental research on the physiology of genetic exchange, particularly by conjugation, is lacking. Gene flow and hybridization can result in modifications in fitness, large fluctuations in population density, alteration in ecological role or function, and changes in host range. Genetic exchange should be characterized in both directions, from agent to native and vice versa, since altered genes may change the activities of the native community, but genes imported from native species to the introduced agent may also cause alterations in characteristics such as competitiveness, pathogenicity, or tolerance to stress.

The Subcommittee agrees that a new direction in risk assessment research should be developed to reorient the research program towards investigation into ecological effects. Prior developments in identification and detection were necessary first steps, but beyond questions of whether the released organism will survive and succeed in the tasks it was designed to perform are evaluations of potential environmental consequences of release. Efforts should be directed to determining the impacts of recombinant organisms on important environmental processes, and the potential for producing ecological or cascading effects. Knowledge gained from making these determinations should be applied by developing protocols and methods for assessing environmental effects, such as microcosm studies and modeling techniques.

Understanding ecological processes is required before judgments can be made about the significance of environmental effects and ecosystem change. Of particular concern is the possibility that introduced genes and GEMs will alter the structure of pathways of biogeochemical processes. Alterations in such fundamental processes as nitrification, oxidation and reduction of sulfur compounds, iron sequestration and chelation, and degradation of bulk organic macromolecules, including cellulose, lignin and chitin, could result in significant environmental effects over time.

Two approaches should be considered in investigating environmental effects. First, from a population perspective, research should be conducted that will permit prediction of the environmental conditions that may allow released organisms to reach large enough population densities to exert an environmental influence. Existing information on plant and insect diseases may provide appropriate examples for application. In addition, several areas should be examined from a research perspective, including: dispersal and growth of organisms in the field, spread of populations in heterogeneous environments, colonization and establishment processes, and the role of spatial scale, temporal persistence and magnitude of release.

The second approach, from a community perspective, involves investigating the role that system structure has in propagating ecological effects. Comparisons of the relationships between macroorganisms and the less well known ecology of microorganisms is a useful starting place. Investigations into the potential for introduced organisms to displace native populations should also be initiated. Introduced organisms become established by one of four mechanisms: 1) developing a new niche with existing organisms, 2) entering a new niche created by environmental stress or perturbation, 3) pre-empting an old niche when existing organisms are absent, and 4) displacing existing organisms to take over a niche. Research is needed to determine whether such displacements occur, which microbial species are likely to be displaced, and the long-term consequences of their loss from the site along with conditions that would promote re-establishment and system resiliency.

Cascading and cumulative effects are among the more difficult environmental effects to predict. When organisms considered to be innocuous, whether engineered or not, are introduced to a new habitat, they are no longer necessarily innocuous. Indirect, cumulative or cascading effects could result as the organisms influence and interact with their new habitat, and as existing species are displaced by introduced ones. Cascading effects, such as those seen in the bioaccumulation of pesticides in falcons and as evidenced by population blooms with phytoplankton, are possible and should be investigated. Cumulative effects may result from numerous, repeated applications as opposed to acute responses to single exposures. The Subcommittee recommends that a component of the program be introduced for long-term, multiple year (3-5) research, to investigate the potential for such effects.

In addition to the research described in the preceding paragraphs, insights into environmental effects that may occur in such situations, their magnitude, and endpoints that are useful measures of such environmental effects can be gained from studying environmental perturbations and disturbances that have occurred in the past. Perturbations such as nutrient enrichment or toxin addition may simulate the immediate effect of an introduced organism. Disturbed or stressed environments may be habitats where the introduction of GEMs has a greater potential to adversely affect ecosystem structure and function, and should be investigated further. The investigations should be aimed at identifying appropriate endpoints for quantification, specifying the interactions that significantly affect community structure, and developing specific protocols for MPCAs that can be used by registrants.

Microcosm studies may provide preliminary approximations of environmental processes and, therefore, have value in assessing and predicting environmental effects. These systems present useful opportunities for standardizing and generalizing conditions for validating experimental analogies and quantifying environmental relationships. The program has made considerable effort to simulate natural conditions in microcosms, and these efforts are supported by the Subcommittee. However, three aspects of microcosm investigations remain problematic: extrapolation to larger size; compensation for greater fluctuation and intensity of environmental influences, such as wind and current, nutrient availability, and temperature; and temporal considerations. Future research should attempt to solve these remaining problems.

Understanding such ecological interactions and effects can also be applied to develop a stronger modeling effort for additional study and prediction. There is a large body of modeling information that has not yet been included for assessing GEMs. Specifically this program should incorporate the body of knowledge on environmental modeling and decision support systems developed at EPA's Environmental Research Laboratory in Athens,

Georgia. The Subcommittee recommends continuing model development to increase the complexity and the predictive capability of models for assessing the environmental impact of GEMS.

In summary, the Subcommittee supports the program's past accomplishments and recommends that the research program be reoriented to determining the impacts of GEMS on environmental processes, and assessing their potential for producing ecological effects. Gene exchange should be investigated to elucidate both the physiological basis and the potential for environmental change that may result. Biological control methods should be developed to control the spread of released organisms and their genetic material. The knowledge gained from these investigations should be applied by developing protocols, methods, and a series of models for assessing and predicting environmental effects. Finally, these methods should be validated and refined by comparison with results from actual field investigations conducted with environmentally released GEMs.

4.1.3 Needs for Health Effects Research

The Subcommittee concludes that the health effects research portion of the program is too narrowly focused and not sufficiently targeted to questions that EPA must address. The reasons for using Pseudomonas aeruginosa are unclear and the use of more relevant organisms is strongly encouraged. More emphasis should be placed on detecting exposure and on developing model systems. Investigations should be conducted to characterize: the ways that microorganisms can transform themselves and other organisms in nature, the means that microorganisms use to propagate and diffuse to potential targets, the mechanisms used to identify potential targets, the susceptibility of target organisms, and the associated routes of exposure. Human exposure as non-targets or through complicated routes, such as exposure of pet species followed by transmission to humans, should be incorporated into investigation strategies.

The Subcommittee's major recommendations include: 1) development of a strategy for conducting research projects more closely targeted to meet EPA's assessment needs, such as model development, detecting exposures by a variety of routes and experimentation with relevant microorganisms, 2) considerable expansion through extramural grants to biomedical or veterinary concerns with appropriate expertise and 3) coordination of this program with those of other agencies experienced in conducting health research, such as the Food and Drug Administration (FDA), the National Institutes of Health (NIH), the CDC, or NIOSH.

4.1.4 Needs for Engineering Research

In the proposed engineering research, which is related to production and uses of GEMs and MPCAs, and is in a very early stage, extensive use will be made of information for the employment, transport, containment, and elimination of microorganisms already available. These data are available from other agencies (i.e. NIOSH), other experimental programs (e.g., Department of Defense, chemical and biological warfare field tests), and industrial experience with microorganisms. This initial dependence is an economical use of abundant information already in existence. The overall research goal is to determine the applicability of methods previously developed for the commercial use of microorganisms to similar application of GEMs.

The research could also make use of two potential advantages available with GEMs but not with conventional microorganisms. First, they can be made more readily distinguishable from wild type microbes by virtue of distinct phenotypes or presence of unique genetic elements identifiable by their molecular structure. Second, those GEMs/MPCAs intended for activity in very narrowly defined sites (e.g., a factory container, fermenter, or a specific target host plant or animal) could be debilitated with respect to general environmental competence or ability to colonize alternative host organisms. In these two areas, the biological research already within the program could guide a further refinement of objectives for the engineering research.

4.2 Additional Recommendations

4.2.1 Training

There is little doubt that biotechnology and genetic engineering methods will be increasingly commercialized in the coming years. Research and development discoveries will move from the laboratory into the market place as products for consumers. EPA will be called on to regulate a diverse group of products, evaluating them from a risk-based perspective. Evaluating such diversity requires staff with broad based training in multiple disciplines.

For years the environmental sciences have competed with the medical and physical sciences for quality students. Biotechnology offers new creative challenges, high technology application possibilities and the promise of a rewarding career, further providing an opportunity to recruit talented students and train future staff in environmentally related disciplines. To further encourage recruitment and training, a significantly increased number of undergraduate summer research grants should be offered to students with promise. Similarly, a number of graduate fellowships in environmental studies should be established that are competitive in training and compensation, and are designed to attract highly qualified students.

These fellowships would permit students to study in the laboratory of their choice and could facilitate cross-training in one or more disciplines to encourage more complete understanding of genetic engineering aspects and opportunities. By recruiting students into the novel technology areas of microbiology, biotechnology, genetics, ecology, and computer science, a program would be created to enhance environmental science pursuits and the quality of associated research, while developing a pool of highly trained staff. Through such programs, EPA may be provided with staff of sufficient breadth to respond to rapidly developing advances in both basic and applied research in these areas, providing the opportunity to effectively incorporate advanced science into Agency programs, regulations and policies.

4.2.2 Soliciting Research Proposals

The Subcommittee recommends that EPA consider re-evaluating the system of soliciting and awarding research proposals. Research program staff indicated that there is a lack of proposals received in certain areas. There is also a possibility that industry is more of a stakeholder in these areas of research. New mechanisms, such as specifically designed contractual solicitations, should be investigated to encourage the development of such research areas, and should involve industrial researchers who have a head start on such developments.

Current grant solicitations may be too broad in scope, although this breadth served a useful purpose in the past. Future solicitations might reflect the use of specific, relevant organisms for which data have not been obtained, or application in field environments as opposed to model laboratory systems. More numerous, smaller grants should be offered with a portion of the research monies, to aid in continued basic research developments. In short, more variety in types of extramural research, including small grants, current types, and industrial contracts to name a few, should be encouraged to meet specific needs.

4.2.3 Links to EPA's Biosystems Initiative

The Biosystems Initiative is a new research program conceived by EPA to investigate the use of microbial or biological agents, both naturally occurring and engineered for pollution control. This initiative is in its initial planning stages, and was therefore not reviewed. Because of the potential scope of the program, and the relationship between biosystems agents and MPCAs in terms of environmental effects, the Subcommittee recommends that the Biosystems Initiative be subjected to independent peer review before final planning and implementation occurs.

4.2.4 Links to Industry and the Scientific Community

Better ties to industry should be established by EPA to ensure that Agency research does not duplicate industry efforts. Good contact has been established with academia; however, industry also conducts a significant amount of biotechnology research. Examples of areas where better external linkages need to be developed include: medical diagnostic research, both nucleic acid and immunodiagnostic; aerosol and atmospheric dispersal research that examines cryptic plasmids; and investigations into gene expression.

Such interaction is currently encouraged by enlisting industry representatives to serve on Biotechnology Science Advisory Committee and Science Advisory Board endeavors. In addition, representatives with appropriate backgrounds should be invited to perform one layer of peer review in the extramural grant review process.

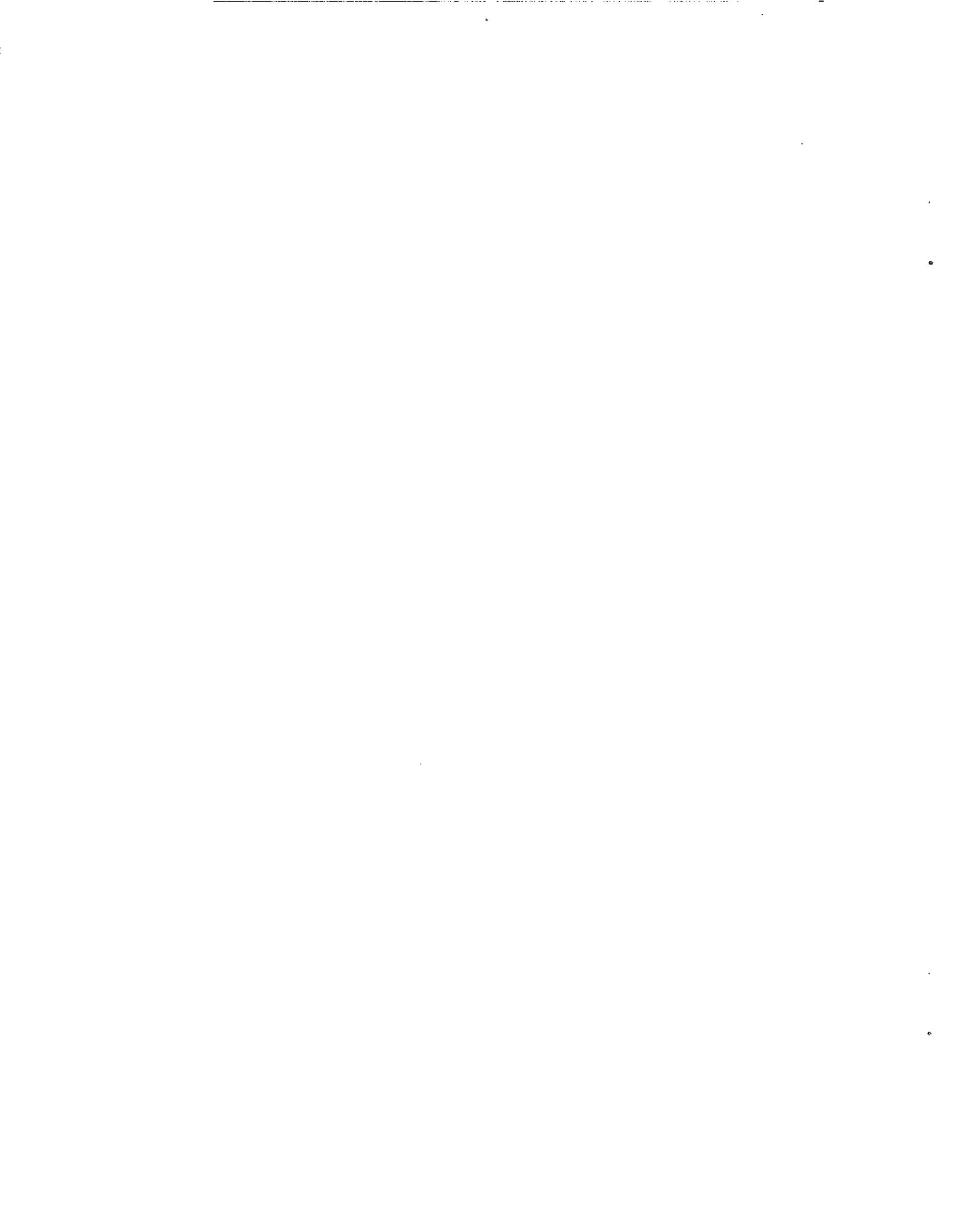
APPENDIX I

Acronyms

CBW	Chemical and Biological Warfare
CDC	Centers for Disease Control
DOD	Department of Defense
EPA	Environmental Protection Agency
GEM	Genetically Engineered Microorganism
MPCA	Microbial Pest Control Agent
NIOSH	National Institute for Occupational Safety and Health
OPP	Office of Pesticide Programs
ORD	Office of Research and Development
OTS	Office of Toxic Substances
SAB	Science Advisory Board

APPENDIX II

STATUS REPORT
FOR BIOTECHNOLOGY/MICROBIAL PEST CONTROL AGENT
RISK ASSESSMENT PROGRAM



ABSTRACT

This document provides a status report of the research program that has been initiated in support of the U.S. Environmental Protection Agency's (EPA) risk assessment of genetically engineered microorganisms (GEMs) and microbial pest control agents (MPCAs) in the environment. The program has been developed in response to the need to produce methods and environmental information for assessing the possible adverse ecological consequences of accidental or purposeful introduction of novel organisms in aquatic and terrestrial systems. Research has been initiated in the areas of organism detection and survival, genetic exchange in the environment, organism dissemination and ecological and human health effects of organisms released to the environment. The program addresses questions relating to the fate and effects of microorganisms used both for biotechnology and microbial pest control. A summary of this research is presented in this document.

The major components of the research program consist of a carefully organized intramural and extramural group of scientists and their associated research expertise from EPA, other government agencies, academia and This expertise spans a wide variety of scientific disciplines including genetics, microbial ecology, plasmid biology, microbiology, biochemistry, toxicology, and pathobiology. Senior staff of the Office of Research and Development's laboratories spearhead the risk assessment program. This includes the determination of appropriate research as well as the orchestration of the extramural program and coordination of research applicable to the specific needs of EPA's program offices.

A biotechnology matrix manager assists in maximizing interactions between scientists and enhancing the application of new scientific information to EPA's risk assessment programs through collaborative studies, workshops, conferences, and peer reviews.

Current research has already produced significant scientific information on several key issues in biotechnology risk assessment. This includes information that preliminarily demonstrates that a) some types of novel organisms and their genetic information do survive for considerable periods in certain habitats; b) a variety of environmental factors control survival; c) the sensitivity of detection methods can be extensively improved d) microcosms have been successfully used to study fate and survival of GEMs under environmentally relevant conditions; e) genetic exchange can occur, by several mechanisms, in different types of habitats; f) novel organisms have demonstrated little tendency to cause ecological effects; and preliminary mathematical models can be developed to predict the fate and dispersal of GEMs and their associated novel genetic elements.

In addition, a number of protocols and methods have been developed which include: a) five interim protocols to test effects of MPCAs on nontarget arthropods; b) three interim protocols to test effects of MPCAs on avian species; c) protocols for use by the Office of Pesticides and Toxic Substances (OPTS) for measuring genetic stability; and d) a monitoring protocol for release of ice minus genetically engineered bacteria.

Most of these results have appeared or will appear in peer reviewed journals and EPA reports. A list of the scheduled outputs of the biotechnology program are given in the text of this report.

FOREWORD

Advances in the genetic manipulation of microorganisms have established the possibility of creating specialized genetic constructs that will be of enormous commercial and sociological importance. The development of the industry has raised the question of the potential adverse environmental effects these novel genotypes might have on ecological processes and on man himself. The novel genotypes are currently considered to be chemicals by the EPA, falling within the regulatory framework of the Toxic Substances Control Act (TSCA) or as pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). As such, registration procedures must be developed to assess the potential risks and benefits of the use of these organisms in the environment. EPA has therefore proceeded to develop both the methods and the scientific information to address these potential problems. This has led to the establishment of a biotechnology risk assessment program.

The overall goal of the biotechnology risk assessment program is to provide methods and guidance for determining the potential risk of accidentally or deliberately released GEMs or MPCAs into the environment. The basic elements of risk assessment research are to develop methods and testing protocols, test the reliability and environmental relevance of the methods and protocols, identify the potential hazards of releasing GEMs into the environment, monitor for adverse environmental effects, formulate and validate the appropriate predictive tools and develop an overall risk assessment strategy. Six areas of research were originally prioritized as essential for risk assessment:

- (1) development of methods for the detection and enumeration of novel organisms in complex environmental samples;
- (2) determination of survival and growth in the environment;
- (3) stability and transfer of the introduced genetic material in the intra- and extracellular environment;
- (4) transport from the point of application or release to other locations;
- (5) detection of adverse environmental response (ecological effects, toxicity, host range change, etc.) to the introduced organisms; and
- (6) host range change.

Each of these areas are currently being investigated by selected research laboratories within the Office of Research and Development (ORD) of EPA. The research strategy has included the development of an in-house scientific staff to provide the proper information data base and the appropriate methods and protocols for risk assessment. The staff scientists have the responsibilities of concurrently developing a complimentary extramural program and fostering interactions and information exchange with scientists in the fields of genetics, biochemistry, ecology and biotechnology. The strategy requires continuing peer and program reviews, reviews of the research in the risk assessment program to maintain high quality research and help in focusing research toward the needs of the program offices.

The ultimate clients for the research originating from the biotechnology program are the Offices of Toxic Substances (OTS) and Pesticides Programs (OPP). In the latter case, a special program was developed for the evaluation of MPCAs. In the near future, the Office of Solid Waste may become a client because of the potential for development of novel genotypes for the bioremediation of hazardous wastes.

Implementation of the research for the biotechnology risk assessment program can be conveniently described in the following manner:

A. Methods for Detection, Identification and Enumeration of Novel Organisms

The requirements for techniques to identify and enumerate novel organisms following accidental or purposeful release is one of the most fundamental prerequisites of the entire risk assessment process. Therefore, the development of one or more combinations of specific, convenient, reliable, and sensitive tracer methodologies, and their associated protocols has been an early research activity. Such methods have been experimentally developed to facilitate the gathering of relevant data by the manufacturer for the regulatory process. A desirable characteristic of any detection/enumeration technique is its wide applicability and suitability for technical application to the detection of any desired novel organism. Identification and enumeration procedures have been designed to discriminate specific novel strains from other organisms present in the environment, even from other strains of the same species.

Agency microbiologists and their cooperative agreement colleagues have employed a battery of criteria and techniques for fulfilling method development needs. Techniques such as "conventional" selective enrichment methods and "modern" molecular approaches, which rely on the fundamental techniques of genetic engineering, have formed the basis of this development. There are advantages and disadvantages of both methodologies, and in the current analyses, a combination of approaches is best able to satisfy the fundamental criteria for identification and enumeration.

B. Methods for Assessing the Fate of Novel Organisms

Test procedures have been designed to determine whether novel organisms

die off, persist or multiply to high levels in different environments.

Experimental studies of the persistence and fate of GEMs and MPCAs in aquatic and terrestrial ecosystems have provided information on biological components of the environment that might become exposed to or affected by the novel microbes. Such information will be valuable in selecting relevant methods and formulating protocols to deal with infectivity, pathogenicity, acute and chronic toxicity, and ecosystem perturbation. Furthermore, persistence and environmental fate studies continue to provide protocols for validating genetic expression in complex ecosystems and delineating biotic and abiotic factors which contribute to survival or nonsurvival of the organism or its genetic traits.

The use of model ecosystems (microcosms) to assess the fate of novel organisms has been predicated on the following practical criteria: 1) the microcosms are flexible in design and can accommodate a spectrum of novel organisms; 2) they prevent accidental release during and after the test period; 3) many types of physical and biotic components of a given ecosystem can be housed in in a microcosm; 4) conditions can be manipulated to determine which factors prolong the survival or encourage growth of the novel organism; and 5) testing protocols can be readily developed for the appropriate client offices.

C. Test Methods for Assessing Genetic Stability of Novel Organisms

Test procedures have been developed to quantitatively measure transfer frequencies of genetic material between novel and indigenous organisms. Suitable systems have been established to monitor the stability of genetic traits in novel organisms. The transfer of genes into new species requires gene pools and new organisms to be monitored. If genetic traits are transferred into microbes which have ecosystem functions different from the

novel organism, new abiotic and biotic components of that system may be adversely impacted if these traits are expressed.

Studies of genetic stability will be largely influenced by results obtained in companion studies discussed in this document. Evaluating concern or risk involving genetic stability, or lack thereof, has been directly tied to results of fate and transport studies. If, for example, it is found that the population of specific novel organisms declines rapidly (hours to days) and does not regrow following entry into the environment, the probability of genetic effects on the environment is greatly reduced. In cases where an organism colonizes an ecosystem and achieves sufficient cell densities to affect other biotic processes, the level of concern for the fate of its novel genetic information rises with greater concern given to the possible impacts or hazards that might result.

Research efforts have been directed toward an all inclusive approach for measuring genetic stability. The approach has considered the myriad of methods by which an organism can be bioengineered or otherwise genetically altered, the kinds of species involved, and the ways in which gene transfer can occur. Physiochemical characteristics of different types of soil, water, and other environmental components that can significantly affect the fate and genetic stability of novel organisms were also considered. Consequently, considerable attention has been focused on studying a broad spectrum of simple microcosms displaying a variety of physiochemical conditions.

The research has been built upon existing information in microbial genetics, DNA/plasmid biology, and a synthesis of fundamental principles of microbial ecology. A multitier approach is currently in use in which experiments proceed from simple, rapid flask experiments to habitats simulated in microcosms. Simple and complex containment systems are being employed

as an approach for assessing hazards to ecological processes. Field studies are also being considered.

D. Methods for Assessing Hazards of Released Novel Organisms

The regulatory goal of permitting the release of only low-risk novel organisms requires the development of test methods that will distinguish between hazardous and nonhazardous organisms, and test methods which allow a determination of the nature of the hazards if they exist. This portion of the research has focused on two major kinds of potential environmental hazards from novel organisms released to the environment: 1) pathogenicity, including toxicity and infectivity for various nontarget organisms; and 2) effects on critical environmental processes.

Our approach to hazard identification is based on the assumption that a variety of test methods can be developed which are applicable to a variety of intended-use ecosystems. The selection of methods has been based on knowledge of an organism's identity, source, unique attributes for which the organism is released, genetic manipulations, and intended use (site, concentration, application method). EPA recognizes the need to develop test methods for pathogenicity, including toxicity and infectivity of novel organisms released in terrestrial and aquatic ecosystems. In addition to pathogenicity and gene stability, there is a need for test methods to determine the nature, magnitude, and consequences, if any, of novel microbes on natural ecosystem processes. Lack of information in these subject areas is currently under investigation in the risk assessment research program.

