

PD-AAV-449
12N 49605

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A.I.D.'s Activities in Biotechnology
Regulatory Considerations

936-1406

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March 20, 1987

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Prepared under contract: DAN-1406-0-00-7008-00
Agency for International Development
ST/AGR
Washington, D.C.

Purpose of the Report

Biotechnology is providing new methods and approaches for addressing difficult problems. It has been a natural progression for A.I.D.'s research portfolio to incorporate these new methods and such activities can be found throughout the Agency. As well as providing enormous potential, the new methods of biotechnology also raise a unique set of regulatory questions. These questions are the subject of intense discussion on the national and international level. It is the purpose of this report to discuss A.I.D.'s activities in biotechnology, specifically recombinant DNA technology, in light of regulatory considerations.

Introduction

The Agency for International Development (A.I.D.) is the principal agency of the United States Government for carrying out the provisions of the Foreign Assistance Act of 1961, as amended. It is committed to help developing countries in their efforts to meet basic human needs -- to overcome problems of hunger, illiteracy, disease and early death.

In devising a long-range development strategy, A.I.D. has reoriented its approach to emphasize four basic programmatic components*:

1. Policy dialogue - When a country requests our help, A.I.D. works with its leaders to design and implement policy reforms that will permit development to succeed.
2. Institutional development and training - A.I.D. encourages and assists with the building of institutions that help the people directly concerned -- and in which they are active participants. These include everything concerned from local credit unions and school boards to democratic selection of leadership.
3. Technology: research, development and transfer - Through both U.S. and host country institutions, A.I.D. promotes technology development directed at third world problems. Special attention will be given to the application of modern research tools, such as biotechnology, to removing major impediments to development.
4. Reliance on the private sector and market focus - A.I.D. encourages governments to place greater reliance on free market forces and the indigenous private sector as the principal engines of sustainable development.

These policy approaches, when applied to the problems of A.I.D.'s emphasis promote self-sustaining development and progress toward shared goals by host countries. (*Blueprint for Development - The Strategic Plan for the Agency for International Development - 1985.)

A.I.D. has long recognized that science and technology are important factors in successful development. Therefore the support of science and technology has been and is an important component of A.I.D.'s strategy. This support serves a dual purpose - solving specific problems as well as supporting the growth and development of scientific capability in the developing world. As a result of these goals, projects funded by A.I.D. contain components not usually found in other research programs (e.g., those funded by NSF). Specifically, in addition to the research itself, A.I.D. projects include training, networking, and technology transfer.

As a development agency, A.I.D. depends on other Federal and private scientific agencies for expertise and advice. A.I.D. has had long term relationships with the National Science Foundation, the National Institutes of Health, the Department of Agriculture and the U.S. Land Grant Colleges and Universities. A.I.D. looks to these scientific agencies for up to date information on scientific advances and developments, assessments of research capabilities, recommendations of technologies and approaches appropriate for developing countries, and scientific expertise for program review and evaluation.

Biotechnology and Research Supported by A.I.D.

The incorporation of the methods of biotechnology into A.I.D. programs has been a natural progression given the Agency's interests in science and technology. Within the Agency the unofficial definition of biotechnology which has been used is very broad and general. It is the technology which develops based on knowledge of the biological sciences. This definition leads to a wide range of activities which fall into the category of biotechnology -- e.g., use of rhizobia for biological nitrogen fixation, plant tissue culture and genetic engineering of animal vaccines.

In light of increasing use of biotechnology generally, and as greater experience is acquired on essentially non-risk activities, it may be that A.I.D. will limit its definition of biotechnology to the aspects of genetic engineering which are of concern. The development of a narrower definition is under discussion at many of the scientific agencies and the Office of Technology Assessment.

Some of the key regulatory questions at issue are, (a) when is an engineered organism considered to be "contained" for experimental purposes (b) what constitutes a "release" of an engineered organism into the environment and; (c) what constitutes a recombinant organism (e.g. What regulatory procedures should apply to an organism which has had specific DNA deleted). These are the issues of genetic engineering more than they are the concerns of a broader definition of biotechnology.

Regulatory Considerations at A.I.D.

A.I.D. is subject to the National Environmental Protection Act (NEPA) with respect to activities which have an impact on the environment of the United States; specific procedures have been developed for considering environmental questions both in the United States and in A.I.D.'s overseas activities. The environmental procedures used to access actions overseas are not required by NEPA but are patterned on NEPA.

A.I.D. has been sensitive to the new types of products resulting from genetic engineering. The Agency has looked to NIH and other scientific agencies for leadership. Regulatory requirements have been incorporated into proposals as it has become necessary, typically citing NIH guidelines and more recently expanding and developing new language to direct the PI to the appropriate Federal agency (e.g., USDA, EPA) as regulations develop.

As the Agency's involvement in this area of research has been evolving, the treatment of these regulatory issues is not uniform throughout A.I.D. (A new project, which uses genetic engineering to develop an improved animal vaccine, serves as a model for addressing regulatory concerns. This project contains a thorough discussion of the issue.)

Due to the overseas components of A.I.D.'s activities another level of discussion is required - ensuring that research activities financed overseas are conducted in a safe manner and with the knowledge of the country in which the activity is conducted. Additionally the consideration of and compliance with the host country's regulations (after they are reviewed to ensure they are adequate) is essential. All actions must also be reviewed in accordance with A.I.D.'s environmental procedures.

A.I.D. is able to achieve compliance with the necessary regulations by defining such behaviour as a condition of financing.

A.I.D. Future Plans in this Area

Standard Requirements

A.I.D. is in the process of developing and applying standard regulatory requirements to its programs. Grants, contracts and cooperative agreements financed with funds made available by the Office of Agriculture are being amended to include the following requirements.

"Compliance with Federal Guidelines and Regulatory Procedures

The recipient will implement this research activity in accordance with:

- a) the National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules;
- b) procedures issued by the USDA, EPA, or other appropriate Federal agency, regarding testing of genetically engineered organisms;
- c) A.I.D.'s environmental procedures; and
- d) such other Federal guidelines and procedures as may apply during the course of research.

Additionally, the cooperator cannot commence testing in any foreign location until written approval for such testing is obtained from A.I.D. and the government of the country where testing is planned. Testing shall be conducted in accord with all applicable regulations of that country.

In addition, however, and prior to commencement of any such testing, the cooperator shall make a judgement and communicate the same to A.I.D. as to whether the regulations, procedures or facilities of the country in question are adequate to ensure testing in an environmentally sound manner. In the event such judgement is that they are not, the cooperator and A.I.D. will consult and agree on the conditions to be applied to the testing which will have such environmental effect.

Reports submitted under this activity to A.I.D. will address regulatory issues as above related to the activity."

Establishment of an Agency-wide Standing Committee on Biotechnology

Activities in biotechnology are spread throughout the Agency. An Agency wide Standing Committee (A.I.D. Biotechnology Committee) is being put in place to serve as a mechanism to centralize the Agency's activity in this area and to ensure that uniform procedures are implemented.

The proposed responsibilities of this committee will be to:

- formalize A.I.D.'s interaction with scientific agencies in this area;
- develop Agency-wide standard provisions for A.I.D. contracts, grants, and cooperative agreements which provide funding for biotechnology;
- develop Agency-wide procedures for handling genetic engineering projects;
- serve as the focal point for all internal and external inquiries in this area; and
- develop procedures for keeping field personnel informed in this area.

A.I.D.'s Activities in Biotechnology

Presented in the following sections of this report are descriptions of the grants, cooperative agreements and contracts funded by A.I.D. which support activities in biotechnology. The activities are presented by Bureau, Office and Mission.

Bureau for Science and Technology - purpose - provide technical support for field activities and develop research activities of worldwide significance.

A. Office of Agriculture Projects:

1. Project title - Improved Animal Vaccines through Biotechnology
Phase I - Rinderpest
Funding Sept. 1986 - Sept. 1989 - \$870,000
Cooperative Agreement with University of California Davis
Principal Investigator - Dr. T. Yilma

The project is designed to develop an improved vaccine for rinderpest, an acute, highly contagious viral disease of ruminants. The vaccine will be developed using vaccinia virus. The primary institution in this project is the University of California at Davis. The U.S. collaborating institutions are the USDA facility at Plum Island and California Biotechnology, Inc. In Kenya the Ministry of Agricultural and Livestock Development, Department of Veterinary Sciences, and the Veterinary Research Laboratory are participating in the project.

The project proposes to develop the improved vaccine and proceed through limited animal testing under contained conditions. No field testing is proposed in this project.

Regulatory Considerations

Since the ultimate goal of vaccine development is use in the field the project documents do discuss regulatory considerations. These are discussed throughout the project paper, the A.I.D. descriptive document. In the cooperative agreement itself the following instructions are given:

"Compliance with Federal Guidelines and Regulatory Procedures

The recipient will implement this research activity in accordance with: a) the National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules; b) procedures issued by the Animal and Plant Health Inspection Service (APHIS), USDA regarding testing of genetically engineered organisms; and c) such other Federal guidelines and procedures as A.I.D. shall deem appropriate.

Additionally, the cooperator cannot commence testing in Kenya or any other foreign locations until written approval for such testing is obtained from the appropriate Ministries of that Government where testing is planned."

This language will be amended to conform to the wording noted under A.I.D. Future Plans in this Area (page 4).

2. Project title: Improved Biological Nitrogen Fixation thru Biotechnology
Approved Funding - Sept. 1986 - 1991; \$4,800,000 S&T;
\$6,275,000 missions
FY 87 Proposed Budget - \$960,000 Actual Budget \$750,000
Cooperative Agreement - U. of Hawaii
Principal Investigator - Dr. B. Bohlool

This is a continuation of a previous project (NIFTAL) in biological nitrogen fixation (BNF). The earlier project, funded from 1975 to 1985 focused on the collection, identification, characterization and production of rhizobia strains useful in the developing world. A large component of this project was training, international trials, networking, information dissemination and technical assistance to further technology transfer.

The new project builds on the rhizobia collection and scientific network established in the first project. It also plans to incorporate more sophisticated research methods including genetic engineering to improve BNF. Specifically the purposes of this new project are as follows:

- increase the efficiency of nitrogen fixing microorganisms adapted to LDC conditions through methods of biotechnology;
- promote the use of BNF in LDCs by assisting them to adapt, use, and disseminate information about BNF; and
- increase their capacity to produce and distribute BNF inocula.

The new project is divided into 5 programs listed below:

1. Genetic technologies for improvement of rhizobium/legume symbiosis for crops and trees;
2. Development of methodologies for monitoring microorganisms introduced into the environment;
3. Environmental data collection to maximize performance of biological nitrogen fixation and to assure optimum use of BNF in cropping systems;
4. Regional resource centers for training, technical assistance and research; and
5. Technical assistance for production of commercial BNF inoculants.

Programs 1 and 2 can use recombinant DNA technology. It is unlikely that Program 1 will result in field testing in the near future. Although much work has been done on rhizobia important in the United States, little genetic work has been done on rhizobia of importance to tropical crops.

Program 2 proposes to use inoculum strains labeled with specific antibiotic markers to facilitate identification and detection. This approach is valuable for studies in LDCs where serological tools are not available. The methods used will include natural selection as well as recombinant DNA techniques.

Regulatory Considerations

The following is found in the project paper (p.45):

"...However, as research progresses, it is expected that new microorganisms will be developed. It is difficult at this time to assess the impact of these microorganisms since their exact nature is unknown. The field testing of such products after development will follow all appropriate Federal and international regulations. The anticipated impact of these products when ready for field testing will be evaluated on a case-by-case basis."

In the substantial involvement section of the cooperative agreement, however, it is stated that:

"A.I.D. will be consulted during the development of annual work plan and has the right of final approval of all areas of work where A.I.D. resources are included." It is also stated that:

"A.I.D. will be involved in the selection of sites, methodologies, and strategies to be used in field activities funded under this agreement."

The following language is being added to the cooperative agreement (cited from page 4):

"Compliance with Federal Guidelines and Regulatory Procedures

The recipient will implement this research activity in accordance with:

- a) the National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules;
- b) procedures issued by the USDA, EPA, or other appropriate Federal agency, regarding testing of genetically engineered organisms;

- c) A.I.D.'s environmental procedures; and
- d) such other Federal guidelines and procedures as may apply during the course of research.

Additionally, the cooperator cannot commence testing in any foreign location until written approval for such testing is obtained from A.I.D. and the government of the country where testing is planned. Testing shall be conducted in accord with all applicable regulations of that country.

In addition, however, and prior to commencement of any such testing, the cooperator shall make a judgement and communicate the same to A.I.D. as to whether the regulations, procedures or facilities of the country in question are adequate to ensure testing in an environmentally sound manner. In the event such judgement is that they are not, the cooperator and A.I.D. will consult and agree on the conditions to be applied to the testing which will have such environmental effect.

Reports submitted under this activity to A.I.D. will address regulatory issues as above related to the activity."

- 3. Project title: Biotechnology for Tissue Culture
Funding: August 1984 - August 1989 \$5,000,000 S&T;
\$500,000 missions;
FY 87 (actual budget) - \$535,000
Cooperative Agreement - University of Colorado 1984-1989
Principal Investigator - Dr. M. Nabors

The purpose of this project is to establish a capability in plant tissue culture research and application in LDCs to overcome constraints to increased production. The project does this through research, the establishment of a functional U.S.-LDC research linkage network, and the establishment of a International Tissue Culture Network and a plant biotechnology training center. This project is a follow-on activity to a much smaller A.I.D. research activity funded from 1980 -1984 at the same university.

The focus of the research in the project has been primarily on plant tissue culture. With the major advances being made in plant molecular biology the project was amended (May 1986) to support limited work in this area. For the proposed amended work the project plans to make use of three specific techniques to produce varieties with improved tolerance to salt and acidity. The techniques proposed are:

- 1. wide crosses by hand
- 2. genetic engineering
- 3. protoplast fusion

This work has just started. Dr. Jim Colbert of CSU is conducting the molecular biology research. His group is attempting to identify proteins that are expressed in salt tolerant marsh grass strains. From the identification of such proteins, the group will work to identify the gene. The testing of a genetically engineered plant is not foreseen in the near future.

Regulatory Considerations

In the original cooperative agreement it is stated that, "It is understood that the University will be responsible for compliance with applicable Federal, State and local requirements relating to activities of the type contemplated under this Agreement (e.g., the National Environmental Policy Act of 1970, as amended, and the Export Administration Act of 1979, as amended.)

The following language is being added to the cooperative agreement (cited from page 4):

"Compliance with Federal Guidelines and Regulatory Procedures

The recipient will implement this research activity in accordance with:

- a) the National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules;
- b) procedures issued by the USDA, EPA, or other appropriate Federal agency, regarding testing of genetically engineered organisms;
- c) A.I.D.'s environmental procedures; and
- d) such other Federal guidelines and procedures as may apply during the course of research.

Additionally, the cooperator cannot commence testing in any foreign location until written approval for such testing is obtained from A.I.D. and the government of the country where testing is planned. Testing shall be conducted in accordance with all applicable regulations of that country.

In addition, however, and prior to commencement of any such testing, the cooperator shall make a judgement and communicate the same to A.I.D. as to whether the regulations, procedures or facilities of the country in question are adequate to ensure testing in an environmentally sound manner. In the event such judgement is that they are not, the cooperator and A.I.D. will consult and agree on the conditions to be applied to the testing which will have such environmental effect.

Reports submitted under this activity to A.I.D. will address regulatory issues as above related to the activity."

4. International Agricultural Research Centers

A.I.D. is one of the international donors which supports the thirteen International Agricultural Research Centers. These centers are located throughout the world and they are coordinated by the Consultative Group on International Agricultural Research. Each center has specific mandates:

- Center Internacional de Agricultura Tropical (CIAT) - Colombia Research programs: cassava, field beans, rice, tropical pastures
- Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT) - Mexico Research programs: maize, wheat
- Centro Internacional de la Papa (CIP) - Peru Research program: potato
- International Board for Plant Genetic Resources (IBPGR) - Italy collection, documentation, evaluation, conservation, and utilization of genetic resources of important species
- International Center for Agricultural Research in the Dry Areas (ICARDA) Syria Research program: farming systems, cereals, food legumes, forage crops
- International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) India Research programs: chickpea, pigeonpea, pearl millet, sorghum, groundnut, farming systems
- International Livestock Center for Africa (ILCA) - Ethiopia Research program: Livestock production systems
- International Laboratory for Research on Animal Diseases (ILRAD) - Kenya Research programs: trypanosomiasis, theileriasis
- International Rice Research Institute (IRRI) - Philippines Research program: rice
- International Institute of Tropical Agriculture (IITA) - Nigeria Research programs: farming systems, maize, rice, roots and tubers, food legumes
- West Africa Rice Development Association (WARDA) - Liberia Research program: rice
- International Service for National Agricultural Research (ISNAR) - Netherlands - Research program: to provide assistance to the developing countries to plan organize, and manage research more effectively

- International Food Policy Research Institute (IFPRI) - Washington, D.C. Research program: to provide an objective analysis of world food problems and to determine those actions and policies that could be adopted by governments and regional and international agencies to effect a continued increase in the quantity and quality of food supplies.

Research at the Centers - Activities in Biotechnology

The centers activities in Biotechnology is predominantly in the area of tissue culture. Diagnostic applications of monoclonal antibodies and DNA probes are also being investigated. The center which is most actively using recombinant DNA technology is ILRAD. It is conducting research on trypanosomiasis and theileriasis in an effort to develop vaccines.

Regulatory Considerations at the Centers

To date, there have been no discussions at CGIAR concerning regulatory issues at the centers. The Scientific Advisor to CGIAR, Dr. D. Plucknett, plans to introduce the topic at the next meeting of the Technical Advisory Committee.

Letters of enquiry were sent to a number of the centers concerning this topic and some of the responses are presented below.

ILRAD:

"Since 1979 ILRAD has been registered with the USA National Institutes of Health Recombinant Advisory Committee and has followed their published guidelines. All current laboratory work is classified as exempt from guidelines taking into account the fact that the classification of T. Parva and T. Vivax as Class 5 agents is the result of USDA regulations excluding the importation of these organisms into the USA, and is not applicable in Kenya, where they are endemic. Future plans include consideration of bench work on modified vaccinia virus as an antigen delivery agent. In addition to observance of NIH guidelines, ILRAD has secured services of expert consultants to advise on any additional containment requirements.

Regarding field work, we have no plans to release genetically altered organism. Should this situation change in context of possible application of virally vectored vaccines, our approach would depend on results of current deliberations of international regulatory agencies."

IRRI:

"This is to address your enquiry about our safety considerations in experiments involving recombinant DNA technology at IRRI. At present our work involving recombinant DNA techniques is strictly done in the laboratory; however, in the near future, we anticipate testing plants with genetically modified microorganisms in the greenhouse. We will not consider carrying out any experiments in the field until environmental safety guidelines are established and approved by the appropriate authority of the host country. We are in discussion with Philippine Institute of Biotechnology who will develop guidelines.

Currently in our work with recombinant DNA molecules or organisms harboring recombinant DNA molecules, we follow, as closely as possible, the "Guidelines for Research Involving Recombinant DNA Molecules-notice" (Federal Register 1986, Vol. 51 No. 88, pp. 16958-16985) set forth by the National Institutes of Health, U.S.A. The specific safety operations being implemented are:

- 1) Routine manipulation and transfer of all microbes containing recombinant DNA molecules is conducted in laminar flow hoods.
- 2) All laboratory utensils that have been in contact with such microorganisms are sterilized by autoclaving.
- 3) Work benches are surface sterilized with alcohol after completion of experiments.
- 4) All inoculated plants are sterilized by autoclaving.

We plan shortly to set up a committee to ensure implementation of safety operations in all laboratories involved in such experiments. Again, the NIH guidelines will be used as a reference to establish appropriate regulations for IRRI, until such time as national Philippine regulations exist.

About our laboratory safety measures for research personnel such as use and disposal of radioactive isotopes and hazardous chemicals, we follow standard guidelines set by IRRI's safety committee, and can forward a copy if you desire."

5. Factors Limiting Biological Nitrogen Fixation

This project is a small grants activity which supports "equal-partner" research directed at improving biological nitrogen fixation. The project has not supported grants including genetic engineering until recently (1985). The project is managed through a Participating Agency Service Agreement with the USDA. Through this agreement A.I.D. provides the funds to support the research grants which the USDA, specifically the Cooperative State Research Service, manages. The USDA is responsible for soliciting proposals, reviewing proposals, grant development and management. In developing the grants USDA is responsible for addressing regulatory questions and requirements.

The two recent grants supported under this project which involves genetic engineering are: "Use of Molecular Biology to Improve Biological Nitrogen Fixation by Bean Rhizobia for Latin America" and "Detection of Rhizobium in Soil, Rhizosphere and Nodules by DNA Probe".

Use of Molecular Biology to Improve Biological Nitrogen Fixation by Bean Rhizobia for Latin America

Funding - \$196,700 for 10/85 - 10/88

Collaborating Institutions : University of Arizona
University of North Dakota
University of Mexico
University of Panama

The grant proposes to identify genes which are responsible for competitiveness and transfer these genes to highly efficient rhizobia. It is likely that this research will not be at the stage of field testing for some time.

Detection of Rhizobium in Soil, Rhizosphere and Nodules by DNA Probe

Funding May 1987 - May 1990 \$90,000

Collaborating institutions - Washington State University
University of Ankara, Turkey

The project proposes to evaluate transposable elements and DNA probes for studying the ecology of Rhizobium in the soil, rhizosphere, and nodules and for potential use as a model marker system for other genetically engineered microorganisms. Detection utilizing DNA:DNA hybridization will be compared to more standard marker methods.

Regulatory Considerations

These grants were reviewed through the USDA, CSRS system and contain an attachment which requires the grantee to follow the NIH Guidelines. The overseas requirements are not clearly stated.

As the new coordinated framework of guidelines is developed CSRS will develop standard language for all grants it manages.

The language noted under A.I.D.'s Future Plans in this Area (page 4) will be added to these grants.

6. Collaborative Research on Special Constraints for International Agricultural Research Centers

This is a new project which also supports small grant activities. The purpose of these grants is to form collaborative research links between U.S. scientists and the International Agricultural Research Centers. The research supported addresses specific problems which are of interest to both parties. The first grants were funded in 1986. None of these grants involve recombinant DNA. There is some work with DNA probes as diagnostic tools.

The grants program is also funded by A.I.D. through the PASA with USDA. Therefore, all grants chosen for funding must first go through the USDA screening procedure and include the regulatory requirements described above for the Factors Limiting BNF.

The language noted under A.I.D.'s Future Plans in this Area will be added to these grants (page 4).

7. Collaborative Research Support Programs

The Collaborative Research Support Programs are a A.I.D./university effort. They are part of the Title XII (BIFAD) program in A.I.D.

There are seven CRSPs in place. Each has a specific focus and is a collaborative effort between a number of U.S. institutions and overseas countries. The CRSPs A.I.D. is supporting are:

1. Bean/Cowpea CRSP
2. Peanut CRSP
3. Pond Dynamics CRSP
4. Small Ruminant CRSP
5. Soil Management CRSP
6. Sorghum/Millet CRSP
7. Nutrition

Several of these CRSPs support activities in biotechnology, predominantly plant tissue culture work. These include the Bean/Cowpea CRSP, Peanut CRSP, and the Sorghum/Millet CRSP. The only program which includes genetic engineering is the Small Ruminant CRSP. This research program aims to strengthen research capabilities of agencies and institutions in the developing countries and the U.S. in small ruminant animal production. The participating institutions include:

U.S.	LDC
University of California, Davis	Brazil (EMBRAPA)
University of Missouri	Peru (INIPA)
Colorado State University	Indonesia (AARD)
Montana State University	Kenya (Ministry of Livestock Development)
North Carolina State University	Morocco (Hassan II University)
Texas A&M University	
Texas Tech University	
Utah State University	
Washington State University	
Winrock International Livestock Center	

The research in this CRSP which involves genetic engineering is under the direction of Dr. T. McGuire of Washington State University. A project has just been started to develop a vaccine for heartwater using genetic engineering. The vaccine to be developed will be a subunit vaccine - a protein product produced probably by E. coli. The initial research is being carried out in Kenya by a Kenyan graduate student who has trained in Dr. McGuire's laboratory. This graduate student is supported by the A.I.D. CRSP.

Collaborators are Dr. S. Chema, director of the veterinary services of the Ministry of Agriculture and Livestock Development of the Kenyan government and Dr. F. Rurangirwa, a full time Washington State University staff member residing in Nairobi, Kenya.

As this work has just begun, field trials of any type are very far off in the future. Additionally the vaccine developed will not be a genetically engineered organism but rather the product of a genetically engineered organism.

Regulatory Considerations

The research that is being carried out in Kenya was reviewed by the Institutional Biosafety Committee at Washington State University.

All CRSP agreements and subagreements are being amended to include the standard language described previously in this report (A.I.D.'s Future Plans in this Area (page 4)).

8. Indo - US Science and Technology Initiative (STI)

This program is a special activity which was started in 1982 following a meeting between Prime Minister Indira Gandhi and President Reagan. Its purpose is to develop closer ties between the two countries through the support of joint research efforts in areas of mutual importance. During the recent visit of Prime Minister Rajiv Gandhi in 1985 the program was extended for another three years

The areas identified for support are:

1. Agriculture Program
 - a) Biological Nitrogen Fixation
 - b) Nitrogen Use Efficiency Research
 - c) Biomass Fuelwood Production Research
2. Health Program
 - a) Blindness research
 - b) Infectious Disease
 - c) Reproductive Physiology
3. Monsoon Research
 - a) Development of numerical models
 - b) Development of systems for acquisition and processing of initial data
 - c) Satellite data processing and archiving
 - d) Research on basic monsoon dynamics

The funds for the U.S. contributions for these activities are provided from a number of different Federal Agencies. A.I.D. supports the programs in Biological Nitrogen Fixation and Nitrogen Use Efficiency Research. Although these funds are provided by the India A.I.D. Mission, the programs are managed by the Office of Agriculture in the Bureau for Science and Technology.

A.I.D. also contributes funds to the Health program which is managed by NIH. NSF serves as the executive agency and the National Academy of Science has the oversight authority for the entire program.

There are four projects in the BNF section under the subtopic Molecular Genetics which utilize genetic engineering.

These projects and the collaborators involved are listed below:

<u>Project Area & Title</u>	<u>U.S. Senior Scientists</u>	<u>Indian Senior Scientists</u>
MOLECULAR GENETICS		
Rhizobia & Azotobacter	Dr. Donald Helinski University of CA San Diego	Dr. H. K. Das J. Nehru University New Delhi
Rhizobium	Dr. Frederick Ausubel Massachusetts General Hospital, Boston, Mass.	Dr. Sushil Kumar Indian Agricultural Research Institute (IARI), New Delhi
Symbiosis	Dr. David Kuykendall U.S. Department of Agriculture (USDA) Beltsville, MD	Dr. Sushil Kumar IARI, New Delhi
Blue-Green Algae	Dr. Robert Haselkorn University of Chicago	Dr. J. Thomas Bhabha Atomic Research Center (BARC)

A description of the collaboration between Dr. Helinski and Dr. Das is presented as an example.

The overall goal of the work is to determine mechanisms by which different bacteria regulate expression of nitrogen fixation genes and to attempt to enhance biological nitrogen fixation using molecular genetic techniques to manipulate genes involved in biological nitrogen fixation.

Four specific areas have been identified for collaborative research.

- 1) Regulation of expression of nitrogen fixation genes in various bacteria that carry out biological nitrogen fixation of major agricultural importance;
- 2) Examination of Rhizobium genes that are essential to the initial steps of bacteria-plant interaction leading to the development of a functional node on the plant;
- 3) Role of phytohormones in biological nitrogen fixation; and
- 4) Development of the firefly luciferase gene as a reporter gene for analyzing gene expression during the development of the plant nodule.

Each country provides funds to their laboratories to support this collaboration. The U.S. laboratories receive limited funds as this program aims to support ongoing research. (Dr. Helinski's group is receiving \$35,000 for 1986-1987 year.)

One of the main goals of STI is to bring the U.S. and Indian scientific community closer together. In order to do this about 25% of A.I.D.'s support for STI (in agriculture) is spent on travel and workshops. The U.S. assumes responsibilities for the costs of Indian scientists travel within the U.S. and U.S. scientists travel to (but not within) India.

Regulatory Language

All agreements and subagreements are being amended to include the standard language described previously in this report (A.I.D.'s Future Plans in this Area (page 4)).

B. Office of Forestry, Energy, and Natural Resources

A new worldwide program has been developed in this Office. The project is named Forestry/Fuelwood Research and Development (F/FRD). Biotechnology has been identified as one of the approaches for investigating global or cross cutting problems.

It is planned that biotechnology, specifically tissue culture and other propagation techniques will be used for the genetic improvement of multipurpose tree species. One quarter of a million dollars for five years has been designated for this work.

The program is not planning to support any genetic engineering or any activities that could result in deliberate release.

C. Office of Health

1. Malaria Research Portfolio - The Office of Health supports a coordinated network of research projects directed at the development of a malaria vaccine. The funding for the entire malaria project for the period 1975 - 1989 is \$75,112,000. This program includes the participation of approximately fifteen institutions. The most current methods are used in these research projects, including genetic engineering.

It is likely that the vaccine developed through this network will be a subunit vaccine - either produced synthetically or by a recombinant. Typically recombinant DNA technology is used to produce specific antigens or DNA probes. No deliberate release of a genetically engineered organism is expected. A brief description of the contracts which utilize recombinant DNA technology and their objectives is presented below.

Contractor - Bio-Medical Research Institute - Dr. M. Hollingdale

Project objectives:

- To achieve the full in-vitro cycle of development of the exoerythrocytic stage of P. vivax and P. falciparum.
- The molecular characterization of the human hepatocyte receptor for plasmodial sporozoites.
- To develop appropriate insectary back-up to produce malaria infected mosquitoes for human volunteer challenge studies.

Contractor - Bio-Medical Research Institute - Dr. Werner Zolg

Project objectives:

- In vitro cultivation of P. falciparum on a scale sufficient to allow the isolation of DNA, RNA, enzymes and antigens.
- Cloning of genomic and cDNA into different suitable vectors and development of sensitive DNA probes to detect the presence of Plasmodium falciparum in infected blood.
- Identification of Plasmodium falciparum strains which overproduce DHFR.
- Purification of DHFR.
- Screening libraries for expressed DHFR genes using polynucleotides as specific probes.
- Sequencing of the DHFR gene from Plasmodium falciparum.

Contractor - University of Hawaii - Dr. W. Siddiqui

Project objectives:

- Production and enrichment of segmenters/merozoites of in-vitro cultured P. falciparum.
- Purification of in-vitro cultured P. falciparum segmenter/merozoite antigens.
- Characterization of P. falciparum merozoite antigens obtained through purified saponin treatment and their comparison with that of natural-released merozoites.
- Vaccination studies of Aotus Trivirgatus griseimembra using a standardized P. falciparum antigen derived from a long-term in-vitro cultured P. falciparum.

Contractor - New York University Medical Center - Dr. Ruth Nussenzweig

Project objectives:

- The synthesis of vaccines using peptides exposed on the surface of CS proteins for the four species of human malaria.
- Development of a chemically defined synthetic anti-malaria vaccine against the several species of human malaria.
- The testing of immunogenicity of the synthetic peptides of the CS protein of P. falciparum, P. vivax and detection of a common blood stage antigen.
- The use of specific DNA probes to detect and quantitate exoerythrocytic forms of malaria parasites.
- The expression of the CS protein gene products of P. falciparum, P. vivax, P. malariae in yeast and any other expression vectors.
- The immunogenicity of the CS protein gene products of P. vivax and characterization of protective blood stage antigens of this parasite.
- The molecular cloning and comparative analysis of the CS protein gene of the human malaria species.
- The identification and characterization of the CS protein of P. brasilianum and comparison of the antigen with the CS protein of P. malariae.

Contractor - Scripps Clinic - Dr. R. Reese

Project objectives:

- To identify, isolate, and chemically characterize the macromolecular components of the asexual erythrocytic forms of human malarial parasites which can be used to induce protective immunity in non-human parasites and ultimately man.

Contractor - Uniformed Services of University of the Health Sciences - Dr. S. Langreth

Project objectives:

- To locate both the surface and internal malaria antigens to which immune animals produce antibodies.
- To characterize those antigenic components structurally, cytochemically, and immunologically.
- To develop assays to assess purity of blood stage antigen preparations and to screen for interstrain antigenic differences and antigenic variations in-vitro.
- To determine the structure and function of the knobs (alterations on the infected erythrocyte surface produce by P.falciparum infection).

Contractor - Uniformed services University of the Health Sciences - Dr. R. Maheshwari

Project objectives:

- Production of highly purified Interleukin 1 (IL 1) and in-vitro assays to determine how best to use IL 1 to achieve optimal human T and B cell responses to synthetic malarial vaccines.
- Generation of antibodies specific for IL 1. Development of IL 1 receptor assays.
- Use DNA technology for cloning and synthesis of IL 1
- Use of interferons and interleukins as immunomodulators for malaria vaccines in primate model systems.

Regulatory Considerations

In some of the new contracts language has been included which instructs the contractor to follow the NIH guidelines. However this language is not in all contracts which use recombinant DNA technology. It is necessary to update all contracts and insert appropriate language.

2. Diagnostic Technologies for Community Health (DiaTECH)

The Diagnostic Technologies for Community Health Program was initiated in September 1985. The purpose of the project is to improve the health status of less-developed country populations through the development, adaptation and transfer of simple, cost-effective diagnostic technologies. The diseases initially targeted for priority attention under the DiaTECH are: malaria, diarrheal diseases, enteric fever, acute respiratory diseases and tuberculosis. The total funding for the program is \$7.25 million for five years.

The project is implemented through the non-profit organization, Program for Appropriate Technology (PATH). Grants are selected and funded on a competitive basis. Some examples are presented below:

Grant title: Development and Evaluation of DNA Probe Technology for the Diagnosis of Malaria in Volunteers - University of Maryland, James B. Kaper

- Funding - \$65,882
- Specific goal - To evaluate various DNA probes in the diagnosis of malaria and compare results with those obtained using thick blood smears and ELISA assays.

Grant title: A Monoclonal Antibody "Antigen Capture" Assay for Diagnosis of Malaria - Diane Wallace Taylor

- Funding - \$135,872
- Specific goals - To develop and evaluate a two-site monoclonal antibody-based assay for the detection of P. falciparum antigens in serum and urine.

Grant title: Development of an ELISA to Monitor Quinine Levels in Malarious Patients - Alister Voller

- Funding - \$19,948
- Specific goals - To develop a practical ELISA test (a simple photometric test with a total test time of 2 hours or less) for the measurement of plasma quinine levels

Regulatory Considerations

The products of this project will be diagnostics. Although recombinant DNA will be used in developing these, none of the final products will be genetically engineered.

There is no language in the grants which directs the grantee to follow the NIH guidelines. It is necessary to update all contracts and insert appropriate language.

C. Office of Population

The Research Division of the Office of Population supports biotechnology research and development programs in the area of reproductive immunology with the goal of developing a contraceptive vaccine. The Office provides very little direct funding in this area. The work supported is predominantly in the areas of biochemistry and immunology. Some activities which use recombinant DNA technology may be supported in the future, e.g., cloning of sperm and hormone antigens. At this time the Office is not supporting any research which will result in the deliberate release of a genetically engineered organism.

As with other Offices of the Bureau for Science and Technology, the Office of Population manages some programs for the Regional Bureaus. Specifically, the Office of Population directs a program for the India Mission on Contraceptive Development and Reproductive Immunology. This program is in the process of being renewed and regulatory issues should be addressed at that time.

II. Science Advisor's Office

The Science Advisor's Office was established in 1981 to support research activities which are more experimental in nature. The office was established in response to Congress which identified the need for developing countries to build institutional capacity to utilize modern science and technology.

The Science Advisor's office supports three research programs. Two of these programs are grouped together under the Program in Science and Technology Cooperation (PSTC).

1. National Academy of Sciences/Board on Science and Technology in International Development
2. A.I.D./Science Advisor's Grant Program
3. U.S.-Israel Cooperative Development Research (CDR) Program

The A.I.D./Science Advisor's Grant Program and CDR Program are managed by the Science Advisor's Office. The BOSTID program is managed through that office at NAS.

1. The first project PSTC supported was a long term grant to the U.S National Academy of Sciences, Board on Science and Technology for International Development (NAS/BOSTID). The grant to BOSTID supports workshops, advisory teams, and study reports. With the A.I.D. grant BOSTID coordinates a number of research subgrants. Well-defined topics are identified through a series of meetings involving both U.S. and LDC scientists. Proposals are then solicited and reviewed. The topics are currently:

1. Grain amaranth
2. Biological Nitrogen Fixation
3. Fast-growing trees
4. Mosquito Vector Field Studies
5. Rapid Epidemiological Assessment
6. Acute Respiratory Diseases in Children

The program which includes activity in genetic engineering is BNF. The NAS/BOSTID committee which reviews all grants has not authorized any field testing. Only laboratory work has been approved.

Regulatory considerations in the A.I.D. funded BOSTID program:

The large overall grant to NAS does not contain any language regarding regulation. The NAS committee in reviewing subgrants has been very stringent - they have not considered any field testing experiments.

It is anticipated that in the Summer 1987 a new overall grant to NAS/BOSTID will be developed. At that time new standard regulatory language will be included.

2. The Science Advisor's competitive grant program is managed by the staff of the Science Advisor's Office. The grants supported are for two to three years and the maximum funding level is \$150,000. Four criteria are used for selection:

- scientific merit
- relevance to development
- innovation
- potential to enhance LDC research capabilities

The grants are made to LDC universities, government laboratories, or the private sector. Grants for U.S. institutions must have a strong linkage with LDC institutions or research needs.

A.I.D./SCI program has identified research modules and premodules:

Biotechnology/Immunology
Plant Biotechnology
Biomass Technology Conversion
Chemistry for World
 Food Needs
Biological Vector Control
Diversity of Biological Resources
Pre-Modules
 Atmospheric, Marine and Earth Sciences
 Engineering
Capacity Strengthening

3. U.S. - Israel Cooperative Development Research (CDR) Program

This is another grant program which is supported and run by the Science Advisor's Office. Administratively, the program is very similar to the A.I.D./SCI program. Grants are awarded to LDC scientists collaborating with Israeli scientists. It is an effort to apply Israeli experience and technical expertise to help solve significant problems in the developing world.

Both A.I.D./SCI and CDR programs follow the same review process. Unsolicited proposals are reviewed by the staff of the Science Advisor's Office with the help of other A.I.D. staff with particular technical expertise. Based on this review requests are made for the submission of full proposals.

The Science Advisor's Office organizes external scientific peer review panels in Washington twice a year to review the full proposals. The peer review panels recommend grants for funding. A grant can be accepted as is, accepted with provisos, resubmitted if there are major revisions, or rejected. The final decision for funding is based on these recommendations as well as the distribution of grants within given countries, and A.I.D. Mission concurrence.

Regulatory Considerations For A.I.D./SCI and CDR Grants

A number of grants funded under PSTC and CDR involve genetic engineering. Throughout the review process both in the preproposal and full proposal stages recombinant DNA and environmental considerations are explicitly "flagged" for further investigation.

In the Science Advisor's Office instructions for the submission of each full proposal, the researchers are requested to submit certification by appropriate institutional committees (in each country involved) that the proposed research meets NIH DNA guidelines or, in the case of foreign countries, national guidelines essentially equivalent to the NIH guidelines. They are also requested to .."specify your plans for containment."

The researchers are also requested to certify that the proposal does not present unacceptable environmental hazards. "All release of exotic and/or genetically engineered organisms most definitely fits within this category."

This certification has been required of all grants since 1984 and is checked by the A.I.D./SCI Review Coordinator. It is also subject to the scrutiny of the external scientific peer review panels used to review full proposals for potential funding.

III. Regional Bureaus and Missions

Typically the Regional Bureaus are just beginning to support activities in biotechnology and this support is predominantly in tissue culture. For a developing country, tissue culture is the most attractive of the "new" technologies. It is not a difficult technology to initiate and it can be used to address several important problems (disease free plants, identification of more stress tolerant strains).

Asia/Near East Region

This region is the most active in Biotechnology. It receives the most grants from the Science Advisor's Office and country missions are incorporating biotechnology into a growing number of projects.

Most country missions have Agricultural Research Projects and/or Science and Technology Development Projects. Often these projects are comprised of subprojects.

India - (Government has established a Department of Biotechnology and is in the process of developing regulations).

India has a large Agricultural Research Project (ARP). Through the Indo-U.S. Subcommittee on Agriculture priority research areas are identified. These areas typically become components of the ARP. The New Delhi A.I.D. Mission puts together a technical team of U.S. and Indian experts (approved by the Indian Committee on Agricultural Research) which develops a project paper and identifies appropriate research institutions.

Two of the components of the ARP are loosely related to Biotechnology. These are:

1. Vaccine Development for Animal Diseases caused by Blood Protista
2. Germplasm Storage

Neither of these projects however, involves genetic engineering. The vaccine development project will use traditional approaches. This work can be easily carried out in Indian laboratories and Indian scientists are familiar with these approaches.

Vaccine Action Program (VAP)

The Vaccine Action Program is another initiative developed during a meeting between President Reagan and Prime Minister Rajiv Gandhi. The purpose of this new program is to "...bring together U.S. and Indian scientists to jointly develop and test new and improved vaccines for immunization against diseases...". The Memorandum of Understanding for establishing this program has been drafted but it has not yet been signed.

One of the components of VAP is a program called Vaccine and Immuno Diagnostics. This program will be the area where the methods of biotechnology are employed. Proposed work includes the development of a rabies vaccine and development of diagnostic probes for malaria. The proposed funding level for the Immuno-Diagnostics Program is six million dollars for five years.

Regulatory Considerations

In the draft of the Memorandum of Understanding Article VII states as follows:

"It is recognized that some of the medical research, such as in the production of antigens, carried out under this agreement will involve Recombinant DNA research.

It is recognized that both countries have similar regulations governing the conduct of recombinant DNA research. It is agreed that all research, involving recombinant DNA technology, will be carried out in accordance with the laws and regulations of the country in which the research is conducted."

The agreement does not make distinctions between bench work and field testing. At this time India is developing regulations for field testing.

Egypt

The Egypt mission has just approved the renewal of one of the components (Science and Technology Cooperation) of its Science and Technology Development Program. This is an eight year project which plans to provide \$8.2 million for Biotechnology and Computer Based Technology. This support includes funds for training and commodities.

Under biotechnology the project plans to support:

- "Techniques relevant to crops from the semi-arid lands, fermentation processes for non-conventional animal feeds, and treatment of industrial wastes."
- "Simple, new techniques, such as single cell processes, tissue culture and protoplast fusion will be employed initially with genetic engineering and recombinant DNA to follow after the fourth year."

One topic mentioned in the project paper which may be a subject for genetic engineering experiments is the enhancement of storage protein in broad beans.

In the environmental analysis section of this paper it is stated:

- "Genetic engineering, planned for the latter stages of STC, will be subject to review with applicable Egyptian, NIH, EPA, or USDA regulations governing this type of experiment as a condition of subproject approval."

The actual contractual and granting documents will be developed by the A.I.D. Mission.

Thailand

Thailand has a large Science and Technology Development Program. Some of the activities to be supported under this program involve genetic engineering, but this is predominantly research. The actual research activities under this program are just being developed and regulatory considerations are under discussion. A.I.D./Washington has requested A.I.D./Thailand to provide information on these discussions.

From the brief survey conducted in the ANE Bureau it appears that most of the other countries in the region are involved only in tissue culture activities.

Latin America

In this region most of the genetic engineering work is supported through the Science Advisor's programs. The country missions support much more applied research.

Africa

The Africa Bureau is supporting limited activity in biotechnology and this involves plant tissue culture. There is practically no activity using genetic engineering.

The Bureau does support two research activities in vaccine development. Research is supported at Tufts University working with Niger to develop an improved vaccine to rinderpest. Specifically the group at Tufts (Dr. Solad) is working to improve the thermostability of the rinderpest vaccine which requires cold storage. The group had discussed the possibility of extending their using genetic engineering but these activities have not been started.

The Bureau is also supporting a project with the University of Florida to develop a vaccine for Heartwater. At this time this work is focused on epidemiology and immunology. This project is funded by the Zimbabwe Mission.