REGULATORY POLICY OPTIONS FOR CANADIAN BIOTECHNOLOGY

A Report for:

Biotechnology Unit
Ministry of State for Science and Technology
8th Floor West
240 Sparks Street
Ottawa, Canada K1A 1A1
(613) 990-6045

Prepared by:

BEAK CONSULTANTS LIMITED
6870 Goreway Drive
Mississauga, Ontario
Canada L4V 1P1

March 1987

REGULATORY POLICY OPTIONS FOR CANADIAN BIOTECHNOLOGY

A Report for:

Biotechnology Unit
Ministry of State for Science and Technology
8th Floor West
240 Sparks Street
Ottawa, Canada K1A 1A1
(613) 990-6045

Prepared by:

BEAK CONSULTANTS LIMITED
6870 Goreway Drive
Mississauga, Ontario
Canada L4V 1P1

March 1987

FOREWORD

This Report was commissioned by the Ministry of State for Science and Technology to provide information in an area that is of interest to Canadians involved in biotechnology and its regulation. The contents of the Report do not represent official views of the Ministry. It is available in French on request.

EXECUTIVE SUMMARY

At present in Canada, there are several private, university and government laboratories that are working on developing biological products, using advanced biotechnologies, that are intended for introduction into the open environment. Several of these products have been or will be in the near future presented to a variety of regulatory agencies for approvals for field testing and marketing. There are approximately 17 different federal and 46 provincial agencies which at the present time see their regulatory mandate embracing one or more particular aspects of biotechnology. The extent to which these regulatory mandates embrace various aspects of the new biotechnologies is unclear in many instances, particularly regarding intentional environmental release of biological wastes and introduction of some biological products. The wide range of potential agency involvement that must be considered, the uncertainty of testing protocols for some areas, and the criteria against which test results will be evaluated creates an uncertain regulatory climate for Canada's emerging biotechnology industry.

The majority of regulatory instruments believed to be applicable to the products and processes of some of the new biotechnologies were initially developed principally for the chemical or traditional agricultural industries. Development of instruments for regulatory control of chemicals was often on the basis of hindsight; when a certain process, chemical or class of chemicals was observed to cause an environmental detriment, appropriate control legislation or regulations were enacted. There is concern, however, that legislation or regulations of this nature, triggered by unacceptable environmental effects, may not be adequate for certain types of biological products which, unlike chemical products, may have the capability of multiplying and spreading in an uncontrolled manner once they are in an unconfined environment. A more effective regulatory control system would begin with pre-release notification, approval, and follow with monitoring designed to answer specific questions. At present, this pre-release notification and screening requirement, along with follow up monitoring, is largely missing from pertinent environmental legislation. Moreover, key environmental legislation that comes closest to meeting this requirement does not cover biologicals, although amendments are currently being considered in this area.

In order to ensure that potential problems do not occur, both from an environmental and public health perspective, and from the perspective of fostering the new biotechnology industry, there is a requirement for a biotechnology overview and regulatory coordination function related to the development and/or application of new biotechnology products. It is suggested that a new non-regulatory office could provide this function. The overview function should include environmental data compilation and information dissemination to ensure that evolving regulatory approaches are consistent among agencies, and reflect the current state of knowledge regarding environmental effects. The present lack of experience, the large uncertainties in environmental risk assessment, and current differences in opinion as to the risks involved in biotechnology suggest that specific biotechnology legislation would be premature at this time. As the knowledge base increases, the need for more specific legislative requirements over and above those of monitoring and coordination may become clear.

In the interim, until case precedents have been established, a case-by-case approach to assessment and approval by regulatory agencies would be appropriate. Prospective case studies of new biotechnology processes and applications would help to more specifically define information requirements and evaluation criteria, to identify weaknesses in legisative instruments, to identify and resolve any interagency jurisdictional problems, and to develop a regulatory road map for industry. During this interim period, it will be very important for the office charged with the biotechnology science overview and regulatory coordination function to be operative at the international, federal and provincial levels.

There is an immediate need for Canada, in conjunction with other countries at the forefront of the new biotechnologies, to begin the assemblage of hard scientific data through directed research studies that will supply the scientific database necessary for the evaluation of specific products as they are brought into the open market. The proposed office would serve as a coordination office to ensure Canadian research in this area is complementary to and not a duplication of other international work.

TABLE OF CONTENTS

1.0 INTRODUCTION

Canada's National Biotechnology Advisory Committee (1984) has defined the term "biotechnology" as "the expansion and application of biological knowledge toward practical ends". According to this definition, we have been living with biotechnologies for many hundreds, if not thousands, of years. Prior to the 1970's, however, the biotechnologies focused primarily on isolation of strains or species of animals and plants from nature, their cross-breeding and selection of the products of those crosses for practical use. More recently, there has been some new technological input into these natural processes involving, for example, the acceleration of random mutation processes in order to select from a more diverse cross-section of progeny, and the development of technologies such as embryo transplantation in order to improve the efficiency of the breeding process.

In the early 1970's, a quantum leap in man's ability to manipulate biological systems occurred. At this time, Boyer and Cohen described a recombinant DNA process whereby one could very precisely remove a portion of DNA at a specific point and insert a piece of synthetic DNA or DNA isolated from another organism. The metabolic function of this inserted DNA would then be expressed in the offspring. Similarly, it was now possible to selectively remove a specific piece of DNA that controlled a particular metabolic process in an organism and thus leave the offspring of that organism incapable of carrying out that process. At about the same time the development of a technology which allowed the fusion of cells from different species occurred, resulting in an uncontrolled mixing of the chromosomal DNA material and the production of offspring which showed some characteristics of each parent. These two new technologies allowed man to very effectively and efficiently cross species barriers that had previously prevented the exchange of chromosomal DNA between distantly related species of higher plants and animals. In nature, the exchange of DNA between distantly related species has been thought to occur only in bacterial populations.

These new technologies and their promise of being able to very precisely engineer new metabolic functions into animals and plants (rDNA technology), and the creation of a much wider range of hybrids (cell fusion) from which desirable attributes could be selected, also accelerated the need for and development of associated engineering technologies that would allow the scale-up and commercial application of these new

products. These new engineering technologies with their roots in the brewing and more recent pharmaceutical industries are being developed at an accelerated pace for application at both the research laboratory scale and at the commercial production scale.

The development of these new biological and bio-engineering technologies is moving at a rapidly accelerating pace from the point where the fundamental 'enabling technology' first appeared on the laboratory scale in the early 1970's to the point where hundreds of products are going to be available for production and marketing at the end of this decade, less than twenty years later. In some cases involving medical diagnostics and drugs where an established approvals process is in place, products of these new technologies are being screened, licensed and marketed. At the present time, however, throughout most of the Western World, many products of these new technologies, which will involve introduction into the open environment, have reached an approvals bottleneck. The means of producing the product have been worked out, but there is not a smooth functioning of an approvals process. There is a high degree of uncertainty about what particular approvals are necessary for which types of product, what protocols must be followed to develop data necessary for approval and what will be the criteria on which the approval or rejection of a product will be based. Until this uncertainty is resolved, and relatively firm research and development costs and schedules through to marketing can be set, it will be very difficult to justify both private and government research spending in these areas.

In Canada, the Ministry of State for Science and Technology has been charged with the coordination of a clear, effective regulatory regime for biotechnology in Canada, which will ensure adequate protection of public health and environment without placing unnecessary financial and regulatory burdens on Canada's newly developing biotechnology industry. A second and necessary part of this objective is to ensure that the chosen approach to regulation of biotechnology is clearly communicated to both industry and the public.

In order to achieve these two principal objectives of defining a clear and effective regulatory structure, and of communicating this structure to industry and the general public, a series of goals must be achieved, including:

1. The development of a set of **policy statements** with which to guide the interpretation and application of laws and regulations in the biotechnology area.
2. The identification of **existing regulatory instruments** at both the federal and provincial level which apply to biotechnology. As part of this process, **regulatory gaps** must be identified along with the appropriate response to fill these gaps.

3. A set of **procedures** must be developed by which:
   - o the establishment and ongoing monitoring of risk assessment and scientific standards can be carried out;
   - o the implementation of stated policies and provision of advice on the appropriate application and interpretation of laws, regulations and guidelines can be based; and
   - o an effective and efficient pathway can be created for industry to follow in applying for approvals.

4. These policies and procedures must be **clearly communicated** to both industry and the public through the publication of a guidebook to regulation of biotechnology in Canada which sets out:
   - o Canadian policy on key issues in the regulation of biotechnology;
   - o an inventory of laws and regulations to be applied to biotechnology at both the federal and provincial level; and
   - o a statement of the procedure to be followed by companies seeking approval to carry out research using the new biotechnologies and to test or market products developed using the new biotechologies.

Within this overall context, this document provides an overview of the science of biotechnology, and addresses many of the regulatory policy and policy implementation questions in this area. A series of possible policy and regulatory approaches that may be applicable in Canada are discussed, and an implementation framework is proposed which is expected to allow clear communication to industry of a coordinated regulatory response in areas of policy implementation.

2.0 THE STATE OF BIOTECHNOLOGY

Advances in our understanding of molecular genetics over the past thirty years have opened new fields of commercial application of biological materials. While traditional applications were confined mainly to harvesting of biological resources (fish, forests, domestic plants and animals), more recent applications include the use of living organisms to process other raw materials, and genetic modification of organisms to improve their value. Some genetic manipulation has been practiced historically by plant and animal breeders; however, molecular genetic techniques, including rDNA and cell fusion techniques developed since 1970, have made possible an unprecedented variety of genetic changes with potential commercial value. These advances in biology have stimulated the rapid development of scale-up engineering technologies whereby micro-organisms produced by these newer technologies and organisms which had been previously very difficult to mass produce can now be produced in commercial quantities. Specific techniques and applications are reviewed in Section 3.0, along with some of the potential risks involved. The development and use of these techniques and their applications is commonly referred to as "biotechnology".

New ventures in biotechnology have arisen over the past decade, and there has been a growing recognition of their commercial potential. Substantial capital outlays are required during the research and development phase, but substantial returns can be realized from new products. Products in the chemical, pharmaceutical and agricultural industries have already reached the market. However, most ventures are still in pre-market stages of research and development or scale-up.

While the number of biotechnology products currently on the market is small, the major developed countries have targeted biotechnology as an economic priority. Development of a favourable economic and appropriate regulatory climate will be critical to the growth of biotechnology in each country. Key elements of the economic climate are venture capital investment, research and development funding by government in strategic areas, tax and investment incentives, intellectual property laws and educational funding. Key elements of the regulatory climate are clarification of project and product approval pathways, information requirements related to approval, and constraints related to environmental or health and safety regulations. Economic or regulatory uncertainties

increase the financial risks associated with new ventures and discourage investment and subsequent innovation.

National and international efforts to define economic and regulatory policy are complicated by the rapidly developing nature of biotechnology. The applications of the next decade can only be vaguely anticipated today, and experience with environmental or occupational risks associated with those applications is almost non-existent. With experience, our understanding is likely to improve, and our economic strategies and regulatory approaches will probably be revised accordingly. Near-term approaches should reflect these uncertainties by being both flexible and appropriately conservative.

The greatest body of experience with biotechnology is in the research laboratory, primarily in medical research. Federal research funding agencies have therefore played a leading role in biotechnology promotion, and development of guidelines and regulations for environmental and public health protection. However, as biotechnology evolves from research and development toward commercial application, and from small-scale easily confined applications toward large-scale operations with increased opportunities for accidental or intentional release of organisms, it moves into areas in which there is much less operational experience and more regulatory uncertainty. It is at this point that agencies with strong regulatory mandates and appropriate environmental experience should become more involved. The high public profile of environmental issues may also require increased public participation in the transition toward new regulatory approaches.

As regulatory approaches to biotechnology are defined, increased separation of regulatory and industrial development functions may be appropriate. The impartiality of the regulatory process may be incompatible with promotion of coordinated national strategies for technological development. Funding agencies may themselves be subject to certain regulatory requirements, setting up potential conflicts of interest. However, notwithstanding this possible separation of function, there is a need for coordination and consistency in both regulation and industrial development policy.

A brief overview of recent international efforts toward development of regulatory approaches and biotechnology policies is presented in Section 2.1. The Canadian situation is reviewed in Section 2.2, with some discussion of key issues to be addressed in formulation and implementation of policy and regulatory structure.

2.1 International Overview

The Organization for Economic Cooperation and Development (OECD) has taken initial steps toward development of an international accord on biotechnology issues, recognizing the need for international cooperation in protection of human health and environment and promotion of international commerce. The OECD brought together experts from member countries to discuss safety considerations for industrial, agricultural and environmental applications of organisms derived by recombinant DNA (rDNA) techniques (OECD, 1986). In limiting these discussions to rDNA techniques, where specific gene fragments are inserted into host organisms, the OECD excluded certain less precise techniques of genetic manipulation such as cell fusion, mutagenesis and artificial selection. However, the OECD noted that its safety considerations may also apply in these contexts.

The OECD document includes a list of safety considerations relating to potential risks of rDNA applications to humans, plants and animals. Potential risk factors to be considered are also listed and discussed in Section 3.4 of this report. However, the OECD does not recommend specific criteria to be used in risk assessment, concluding that it is not yet possible to develop data requirements or assessment criteria. The OECD recommends a case-by-case approach to risk assessment, by which it means that specific criteria would be established for each new proposal evaluated. Presumably consistent criteria would evolve with accumulation of experience in evaluation of proposals, although the degree of consistency achieved would probably depend upon the nature and stability of the regulatory infrastructure in member countries.

The OECD also recommends a step-by-step assessment process, moving, where appropriate, from the laboratory, to the growth chamber or greenhouse, to limited field testing and finally to large-scale field testing, with risk assessment at several stages. A notification scheme is suggested as a possible means of ensuring that rDNA applications, particularly in environment and agriculture, are subjected to appropriate assessment.

The World Health Organization (WHO) held an international meeting in 1982 to establish a concensus on potential health impacts of biotechnology. The publication based on this meeting (WHO, 1984) provided a good review of biotechnology processes and applications, but the discussion of health and environmental risks was limited. Three very general assessment criteria were proposed, including:

- o capacity of proposed work for adverse effects,
- o probability of organisms escaping, and
- o safety of products and handling methods.

More specific criteria would presumably be established on a case-by-case basis.

Consideration of pathogenicity, allergenicity and/or photosensitivity, hypersensitivity, toxicity, carcinogenicity, mutagenicity and/or teratogenicity was recommended as part of the risk assessment process. Routine worker surveillance and monitoring programs, and documentation of safety, containment and organism identification procedures were also recommended. However, WHO suggested that pathogenicity was a complex trait, unlikely to be transfered accidentally between organisms in rDNA research, and even less likely to appear unexpectedly in a production strain.

The European Economic Communities (EEC) adopted guidelines for rDNA research in 1982. These were adopted as non-binding recommendations to the 12 member states. The EEC guidelines stipulate no stricter conditions on rDNA research than those already existing in any member state. They recommend notification of appropriate government authorities before conducting rDNA research, but do not recommend requirements for prior government approval of the work. General information requirements for notification are specified and it is suggested that all information be treated confidentially. Among the issues addressed by most national guidelines, but not by the EEC, is the question of whether industry as well as government is intended to comply.

A review of European health and safety regulations appropriate to biotechnology has been prepared for the U.S. Office of Technology Assessment (Fox et al., 1983). Walgate (1985) summarizes the state of biotechnology regulation in Europe, noting great differences in the availability and intent of appropriate legislation. While guidelines exist in all 12 EEC states, notification is intended to be mandatory for industry only in Sweden and Germany. Enforcement mechansims vary, but are generally weak or non-existent.

In the United Kingdom, guidelines established by the Genetic Manipulation Advisory Group (GMAG) are intended to be voluntary, except for experiments involving plant pests

or field experiments. The guidelines stipulate mandatory notification and licensing of experiments involving genetic manipulation of plant pests, and require approval of any field experiments by the Health and Safety Executive.

In Switzerland, a self-regulation system administered by the Commission for Experimental Genetics, under the Swiss Academy of Medical Sciences, appears to have worked as well as any government-administered system in ensuring compliance with rDNA guidelines. The voluntary guidelines in Switzerland are identical to the 1982 National Institutes of Health (NIH) guidelines in the U.S.

In the Federal Republic of Germany (FDR), 1978 guidelines based on the NIH model, but with more requirements for personnel monitoring and training, are administered by a Central Commission on Biological Safety (CCBS) within the Ministry of Research and Technology. The CCBS attempts to retain a role in supervision of research, in contrast to the system of local supervision in most other countries. CCBS approval is required for large-scale work involving more than 10 L of culture. While the guidelines are intended to be mandatory, the only enforcement device until very recently has been through control of government funding.

Recent events in Germany described by Dickson (1986) suggest that stringent guidelines must be either legally binding or effectively voluntary. Commercial companies claim to be disadvantaged by FDR guidelines in comparison to foreign competitors. Gen-Bio-Tec, a small Heidelberg firm, apparently conducted experiments on microbial production of human blood-clotting factors without notification of the CCBS. With the 'Green' party in parliament expressing opposition to all industrial uses of genetically engineered organisms, and an all party commission of inquiry due to advise on liberalization of guidelines, the Gen-Bio-Tec infraction has prompted the Minister of Research and Technology to announce that revised guidelines, though they may become more liberal, would soon be legally binding on industry.

The Commonwealth Government of Australia in 1981 established the Recombinant DNA Monitoring Committee (RDMC) within the Department of Industry, Technology and Commerce. The role of the RDMC is intended to be primarily advsiory and logistical. It would receive and monitor applications for proposed rDNA projects, conduct a preliminary evaluation of each proposal, and pass proposals for environmental release of

modified organisms on to appropriate regulatory agencies. It would also respond to requests from regulatory agencies for information on other techniques of genetic manipulation, and may be involved in certification of laboratories for rDNA work. It has produced guidelines for small-scale (laboratory) and large-scale (scale-up and production) work. Industry is expected to follow RDMC guidelines and any additional RDMC recommendations related to specific proposals.

The RDMC has published a consultation document outlining its proposed role in evaluation of proposals for release of recombinant organisms (RDMC, 1985). This document includes proposal submission procedures and specific information requirements relating to potential risks of environmental release. Any information designated as confidential by the proponent is subject to confidentiality legislation precluding disclosure by any member of the RDMC.

Japan adopted a version of the U.S. NIH rDNA guidelines in 1979. Like the NIH guidelines themselves, the Japanese guidelines have been relaxed since that time, although biological containment measures are more strict than those required by NIH. Two sets of guidelines are administered, one by the Ministry of Education applicable to university research, and one by the Science and Technology Agency applicable to national research institutes and business corporations. They prescribe case-by-case evaluation of proposals for environmental release, although no evaluation criteria are specified, and no such proposals have been received (Itoh, 1985). The Ministry of International Trade and Industry is currently drafting a third set of guidelines governing industrial fermentation.

The U.S. National Institutes of Health (NIH) set up a Research Advisory Committee (RAC) in 1976 with multiagency representation to draft guidelines and review proposals for rDNA research funded by the NIH. The NIH guidelines prescribed various containment levels for different types of rDNA research, depending on the organisms used and their degree of pathogenicity. They have been widely adopted with minor modifications by other countries since 1976, but apply only to laboratory research.

In 1982, NIH received an application to field test the 'ice-minus' bacterium, a recombinant strain of Pseudomonas syringae which colonizes plant leaves and protects them from ice formation at low temperature. In 1983, the RAC approved the application and the NIH was sued by Jeremy Rifkin and a coalition of environmental groups for not

filing an environmental impact statement as required by the National Environmental Policy Act (NEPA). In 1984, a U.S. District Court order stopped RAC approval of field testing under NIH research grants.

In 1984, the RAC approved several industrial proposals for field testing, one from Advanced Genetic Systems (AGS) involving the 'ice-minus' bacterium. The project was not NIH funded, and was submitted voluntarily to the RAC. This approval was challenged by Rifkin, but upheld by the Federal court.

Meanwhile, in 1983, the U.S. Environmental Protection Agency (EPA) announced its own authority over environmental release of rDNA organisms under the Toxic Substances Control Act (TSCA) and, in 1984, announced a 90-day pre-notification requirement for field tests of rDNA pesticides. Its first application, from Monsanto, was for field testing of a recombinant Pseudomonas fluorescens containing a gene for an insecticidal toxin from another bacterium Bacillus thuringiensis. EPA required additional information and permission to disclose this information in support of its ultimate decision. After some initial resistance, Monsanto has complied with the EPA request, and a decision is pending (Sun, 1986a).

In 1985, EPA approved an application from Mycogen to field test a pesticidal formulation similar to Monsanto's, but with the recombinant organism killed. EPA declared that killed recombinant pesticides required no special permits. EPA also approved an AGS application for 'ice-minus' field testing (Sun, 1985). McCormick (1985) reviews the recent history of biotechnology litigation in the United States.

Following EPA approval of the AGS field test in Monterey County, California, county authorities held a public hearing and, in the force of strong local opposition, indicated that they would not permit the experiment. EPA was criticized for failing to give adequate notice to the county and for not having visited the site of the experiment (Sun, 1986b). Finally, EPA learned, through an allegation by J. Rifkin, that AGS had conducted previous tests on its roof rather than in a contained greenhouse as implied in its submission to the EPA, and proposed a fine of $20,000.00 for falsification, and suspended the AGS field testing permit (Sun, 1986c; Crawford, 1986a). The EPA, upon completing an audit of AGS operations, later dropped the charge of falsifying data, and the associated fine, and faulted AGS for inadequate reporting. This example emphasizes the point that, where there are uncertainties in what types of data need to be reported and the criteria

for evaluating the data, negative publicity can result for both the biotechnology industry and the regulatory agency concerned.

The U.S. Department of Agriculture (USDA) entered the regulatory arena quietly, approving an rDNA vaccine against pseudorabies in livestock, first for field testing in 1985 and then for marketing in 1986. The vaccine, developed by Biologics Corp., contains living herpes viruses, although their infective and reproductive capabilities have been genetically deleted. The USDA has come under recent criticism for not alerting state authorities to the nature of the field trials, and for not conducting an environmental risk assessment. In response to a petition filed by Rifkin, the USDA suspended the marketing license pending documentation of its risk analysis (Jaroff, 1986). However, the only biotechnology litigation against the USDA concerns its own rDNA research. Rifkin challenged the USDA mammalian gene-transfer program in a 1984 court action (Fox, 1984).

While the decisions of regulatory agencies are challenged in the U.S. courts, the agencies themselves have disagreed on jurisdictional boundaries. Concern was expressed both within industry and government that this would delay research and discourage applications. For example, AGS felt obliged to submit its application twice, once to the NIH and once to the EPA, a process which took two years. In an effort to resolve jurisdictional problems, a Biotechnology Science Board (BSB) was proposed as a second-tier review agency (Culliton, 1985). However, it appeared that this would either undercut the authority of EPA, NIH, FDA and USDA, or duplicate their effort, putting industry in a 'double-jeopardy' situation (Rhein, 1985). A Biotechnology Science Coordinating Committee (BSCC) within the Office of Science and Technology Policy (OSTP) was then proposed as an alternative coordinating forum to promote consistency among regulatory agencies, clarify jurisdictions and conduct generic reviews (Federal Register 50:220, 1985). Legislation to establish the BSCC has been introduced to Congress (Crawford, 1986b). Effective coordination should serve to foster public confidence in biotechnology as an emerging industry.

The Canadian Medical Research Council (MRC) established guidelines for rDNA research in 1977. These were modelled after the U.S. NIH and U.K. GMAC guidelines, prescribing six physical and four biological containment levels for different types of animal viruses and cells according to their taxonomy and pathogenicity. The MRC guidelines have been updated twice, the latest revision in 1980 (MRC, 1980). They are designed for small-scale

laboratory work and do not address deliberate environmental release of genetically engineered organisms.

In 1980, the Ministry of State for Science and Technology (MOSST) established a task force to advise on an effective strategy for development of biotechnology. On its advice, the Canadian government adopted a National Biotechnology Strategy (NBS) in 1983, and established a National Biotechnology Advisory Committee comprised of members from industry, government and universities, to advise the Minister of MOSST on industry developments and policy needs. A Federal Interdepartmental Committee was also established to review federal government activities and monitor the NBS. The Interdepartmental Committee established a Working Group on Safety and Regulations in 1985, co-chaired by Environment Canada and the Department of National Health and Welfare. Subsequently, a smaller sub-group on safety and regulations was formed, chaired by Environment Canada, which now serves as an inter-agency coordinating committee.

On behalf of the Working Group, Environment Canada has recently completed an inventory of existing regulatory instruments at federal and provincial levels which may be applicable to regulation of modern biotechnology (Henley, 1986). It was apparent from this inventory that uniform standards across federal and provincial jurisdictions would be very difficult to achieve on the basis of current legislation. Most of the regulatory instruments identified by federal and provincial agencies as applicable to biotechnology were environmental statutes. However, provision for sanctions and penalties on violation were generally lacking.

Miller (1986) briefly reviewed some of the key federal legislation. The Environmental Contaminants Act (ECA), jointly administered by Environment Canada and the Department of National Health and Welfare, is limited by a specific statement of applicability to inanimate chemical - not biological - substances. Moreover, it lacks requirements for pre-manufacture or pre-market notification by industry. The Pest Control Products (PCP) Act, administered by the Department of Agriculture, and the Food and Drug Act (FDA), administered by the Department of National Health and Welfare, both prescribe pre-market notification, testing and departmental evaluation, although the departments may have to change their data requirements to effectively evaluate genetically engineered organisms. Both the PCP Act and the ECA are scheduled for review in the near future. At the present time, many biological products

(e.g., seeds) and most biological wastes intended for environmental release are poorly covered by federal legislation designed for environmental and human health protection.

Recent experience in other countries, notably the U.S. and FDR, suggests a great potential for public controversy and legal confrontation in the commercialization of biotechnology. It is clear that new regulatory approaches are required to deal with new potential risks and concerns. In Canada, an opportunity exists to avoid confrontation by promotion of constructive dialogue at an early stage. Success in this objective depends on identification of key issues before they become polarized, and establishment of flexible and responsive governmental structures for dealing with these issues on an immediate interim basis and over the long-term.

2.1.1 Key Issues in Biotechnology

International experience in biotechnology over the past several decade illustrates a number of key issues which have been subject to public debate and legal controversy. Public concerns have focused on protection of human health and the environment. Legal controversy has focused on questions of jurisdiction and authority in biotechnology regulation. Specific cases give rise to broader questions of policy and implementation.

In the United States, the 'ice-minus' case, for example, illustrates public concern over ecosystem perturbation, a specific concern in this case being possible interference with local water cycles by the 'ice-minus' bacterium. It also illustrates the jurisdictional problem, with two separate agencies competing for federal authority and local government denying permission for federally approved field tests. The P. florescens/B. thuringiensis case illustrates concern over environmental toxicity and possible adverse effects on beneficial organisms. It also raises a number of scientific questions concerning legal treatment of dead vs. living recombinant organisms, and organisms with and without foreign DNA, as well as industry concerns over release of proprietary information by regulatory authorities.

The specific resolution of some of these questions may require considerable study as experience accumulates in dealing with biotechnology applications involving planned or accidental environmental introduction of engineered organisms. However, interim decision-making mechanisms are required now, along with mechanisms for continually monitoring our experience with new biotechnologies and evaluating governmental

responses. In order to provide a mandate for establishing these mechanisms and investigating some of the more specific questions relating to regulatory approaches, a biotechnology policy statement is now required. Some of the broader policy issues which should be addressed in such a policy statement are discussed in Section 2.1.2. Specific issues related to implementation of policy are discussed in Section 2.1.3.

2.1.2 Regulatory Policy Issues

The confusion surrounding recent environmental ventures in biotechnology suggests that the regulatory policies and policy implementation procedures which have been developed for other technologies are not adequate for these newer technologies. In order to develop regulatory approaches and industrial strategies that are effective in the specific context of biotechnology, it is first necessary to explore a range of broad policy questions. The following paragraphs address seven broad policy questions, with a brief discussion of policy options in each area.

1. What should be the nature of federal policy statements in the area of biotechnology at the present time?

Two policy options consist of:

a) specific policy stated in very precise terms, leaving little room for interpretation, and
b) general policy stated in loose terms such that there is considerable flexibility in its interpretation.

The first option requires considerable understanding of the underlying science of biotechnology and its environmental and societal impacts. The second option merely requires a concensus on societal objectives, while permitting sufficient flexibility for regulatory bodies to develop specific approaches to implementation.

The second option would appear to be most appropriate for biotechnology at the present time, considering the many scientific uncertainties involved and our lack of experience with environmental applications of the new biotechnologies. The uncertainties include identification and quantification of risks to human health and environment, and methods

and information requirements for risk assessment. These are discussed in greater detail in Sections 3.4 and 3.5.

Objectives identified as part of a general policy statement could include protection of human health and environment from risks associated with biotechnology, and/or promotion of biotechnology research in certain areas deemed important to the Canadian economy.

2. a) Are the new biotechnologies currently being developed fundamentally different from other technologies in a way that would require a policy statement specifically for biotechnology? and

   b) Should all biotechnologies or their products be treated the same way?

These questions are related in that both pertain to the definition of biotechnology and its unique characteristics. Various definitions of biotechnology have been used. Narrow definitions restrict the term to the use of recombinant DNA techniques where specific pieces of foreign genetic material, either synthetic or derived from another organism, are inserted into a host organism. Broader definitions include cell fusion techniques where the total complement of genetic material from two organisms is mixed to form a new type of cell. Still broader definitions include random mutagenesis and artificial selection techniques which have been used for centuries by plant and animal breeders.

All three biotechnologies are fundamentally different from other technologies in that they involve production of new life forms, although it is usually difficult to prove that the new life form does not already exist in nature somewhere. The behaviour of a new life form, or an old life form placed in a novel environment, is difficult to predict. Various perturbations of the environment are possible and some may adversely affect man. The ability of biological organisms to reproduce and multiply raises the possibility that broad geographical areas could be irreversibly affected.

The three biotechnologies differ from each other in their precision. While recombinant DNA techniques allow genetic exchange between very distantly related organisms, small quantities of specific genetic material may be excised and transferred with great precision. Cell fusion allows a greater degree of genetic exchange, and more opportunity for unexpected genetic changes. Mutation/selection techniques allow a broad spectrum of genetic changes to occur within a single species, but have a long history of use without

serious consequence. Thus, there is a rationale for different regulatory treatment of each biotechnology process, based upon both the degree of precision associated with the technique and our collective experience of dealing with the products of these technologies.

Since there would be considerable difficulty in forcing a new policy or regulatory approach on long-established technology, such as mutation/selection, and since historical experience enhances our confidence in the safety of this technique under present policies and regulatory structures, it may be prudent to limit a policy statement to the new biotechnologies - recombinant DNA and cell fusion - which have been the focus of recent public concern.

3. Should government policy focus on a reduction of environmental and public health risks, or on promoting development of biotechnologies in order to realize their benefits?

These policy options are not incompatible and, in fact, any balanced policy statement should acknowledge both potential risks and potential benefits. Nevertheless, a policy which gives priority to one of several objectives is typically easier to implement than one which lacks such prioritization, since there are inevitably cases in which two objectives are perceived to be in conflict. Giving priority to risk reduction gives a stronger regulatory mandate, and a greater assurance to the general public that their concerns are adequately represented. Also, it is in the ultimate interest of biotechnology industries to maintain such public confidence.

Assignment of priority to risk reduction gives regulatory agencies the option of insisting on use of best available technology to minimize risk to the public and the environment. This authority should probably be balanced, however, with a statement of intent to allow the orderly development of biotechnology in Canada.

4. a) what areas of biotechnology require some level of government protocol development and evaluation, and
   b) what aspects of protocol should be subject to this review?

Areas of biotechnology may be defined in terms of fundamental processes of genetic engineering, such as recombinant DNA and cell fusion techniques, engineering scale-up

processes, such as fermentation systems or in terms of broad categories of application, such as crop improvement, pest control or enhanced degradation of waste. Certain areas may be better covered by existing legislation or protocol than others.

While MRC guidelines provide standards for conduct of contained laboratory experiments, enforcement mechanisms may be needed with increased availability of private funding and reduced powers of persuasion by funding agencies. Standards are lacking for field trials involving open environmental release of engineered organisms, as are clearly defined approval pathways to be followed by industry in obtaining permission for such experiments from government. A policy statement should identify (a): the federal role in the funding of production and field release protocol development in order to assess risk, and (b) the federal role in the development of criteria for the evaluation of protocol data in deciding levels of acceptable risk for any particular area of biotechnology.

5. Is there a need for a notification requirement in all or selected areas of new biotechnology, or at certain stages of product development?

The advantage of a notification system is that it enables government to monitor activities and consequences in designated areas of biotechnology, thereby building a body of knowledge on which to base future risk assessment. It also provides the opportunity for intervention in projects which lack adequate safeguards for human health and environmental protection. Understanding of adequate safeguards and risk assessment procedures will increase with the expanding national and international knowledge base.

Notification may take place at the research and development stage, prior to scale-up or field testing, or prior to product sales. Since the advisability of proceeding to each new stage may depend upon findings at previous stages, a multi-stage notification process may be required. Mechanisms for enforcing notification and defining conditions for approval of work at each stage are also necessary.

6. Are there any areas of biotechnology which require fiscal support or outright prohibition?

Promotion of biotechnology in areas considered critical to Canada's economy may be necessary in the early stages of industrial development when considerable research effort

is required and before investor confidence has been established. The question of whether and where to direct limited fiscal resources for maximum advantage should be carefully considered. Selecting certain areas of biotechnology for preferred treatment may tend to discourage innovation in other areas, and may lead to lower standards than a system based entirely on proposal merit. On the other hand, support diffused in many directions may have little impact.

Prohibition of certain fields of biotechnology research may be considered on ethical grounds or on the grounds that they involve unacceptable risks. Activity in areas, such as, for example, germ warfare research, could have adverse impacts on public perception of other research areas and on general investor confidence.

7. Is there a need for a full and open public debate on the various issues of policy and implementation related to biotechnology in Canada?

Public participation may be incorporated into the processes of policy development, development of implementation procedures, or specific project assessments. Involvement of the public at an early stage may foster public understanding of the issues involved, encouraging rational analysis as opposed to emotional reaction. It may also serve to assure the public that their concerns are adequately represented by government.

On the other hand, the complexity of the scientific issues and the uncertainties involved in risk assessment may not be particularly reassuring to the public. Public education, and coordinated government action toward a cautious and flexible mechanism for biotechnology regulation, are necessary elements of a constructive public dialogue.

Members of the 'public' who may have a role in specific project assessments should have a detailed familiarity with the scientific issues and a continuing involvement in project assessment. This does not necessarily preclude members of the lay public, although substantial time commitments would be necessary. Continuity of membership in assessment committees would be essential to ensure consistency in application of project evaluation criteria.

2.1.3 Implementation Issues

Many specific issues related to implementation of a regulatory policy for biotechnology will have to be addressed by government departments and coordinating agencies charged with implementation. These issues include technical questions requiring expert study and continual reassessment as new information based on experience becomes available. Therefore, it would not be appropriate to entrench interim solutions in a formal policy statement. Some implementation issues, which are currently being addressed internally by regulatory agencies in Canada and other countries, are listed and briefly discussed in this section.

1. What risks associated with biotechnology need to be assessed in order to protect human health and environment, and what are the best methods of estimating their magnitude?

These two related questions follow from Policy Issue No. 4. The need for a risk assessment protocol to assess the risks associated with release of engineered organisms has been identified in a number of policy documents prepared for and by the U.S. government (Arthur D. Little Inc., 1984; EPA, 1986). The OECD (1986) has compiled a list of scientific considerations that may be useful in biotechnology risk assessment, but quantitative data, methods of obtaining such data and guidelines for drawing inferences from such data are needed. The Massachusetts Institute of Technology Centre of Technology Policy and Industrial Development has suggested a number of microcosm test methods and modelling approaches that seem to be appropriate, but emphasizes that the science of biotechnology risk assessment is in its infancy (Strauss et al., 1986). The costs of developing this science will be considerable, and should probably be borne by government. Risk considerations and risk assessment methods are discussed in more detail in Sections 3.4 and 3.5.

2. Would proprietary information be required from industry in order to conduct a risk assessment, and how can confidentiality of such information be ensured?

While specific information requirements have yet to be defined, it is likely that, at some stage prior to introduction of a product into the open environment, proprietary information will be needed. There may be pressure on regulatory agencies to release this information, at least in the first few cases considered, in order to assure the public that

information, at least in the first few cases considered, in order to assure the public that an adequate assessment has been performed. The U.S. EPA, for example, recently demanded and obtained permission to release proprietary information supplied by an applicant. If mechanisms to ensure confidentiality cannot be devised, important projects may be postponed by industry or developed elsewhere.

3. What level of risk should be considered acceptable by regulatory agencies, and how should this level be determined?

Value judgements of this type are implicit in any regulation decision. Risks accepted in other industries may be used as the standard; however, there are inconsistencies among other industries. Perceived as opposed to actual risk is often a deciding factor, resulting in possible overregulation of certain industries. Voluntary risk is often perceived to be more acceptable than involuntary risk. Explicit guidelines for making such value judgements would encourage consistency in regulatory approach.

4. Should 'new' organisms be treated differently than organisms produced by genetic engineering, but known to occur naturally, and how should a new organism be defined?

The premise that novel organisms are inherently more dangerous than naturally occurring organisms requires expert scrutiny. A more rational approach to estimating risk may be based on specific criteria of organism function, genetic background, and degree of genetic and functional definition. Such criteria are discussed in greater detail in Section 3.4. However, if novelty is used as a criterion, the term will have to be defined. A certain degree of variability is characteristic of any biological species. While previously unknown functional characteristics representing a qualitative change can logically be considered new, quantitative changes in function would have to be shown to deviate significantly from the norm, by statistical or other criteria. Alternatively, any known genetic alteration, regardless of functional change in the organisms or natural variation within the species could be considered to create a new organism.

5. Should organisms containing foreign DNA (from other species) be treated differently for regulatory purposes than other genetically altered organisms?

There is no evidence that the potential for unexpected adverse effects is greater in organisms containing foreign DNA. However, it is probably true that the spectrum of possible genetic changes is increased by the use of foreign DNA. Logically, risk evaluation and regulatory treatment should be based on a knowledge of genetic makeup and functional performance. The precision with which foreign DNA can be inserted in a recombinant organism permits a greater knowledge of genetic makeup than do some traditional practices of plant and animal breeding.

6. Should dead organisms in a product be treated differently than any chemical ingredient for regulatory purposes?

Dead organisms lack the capacity for reproduction which calls for a regulatory distinction between biological and chemical products. However, other living organisms in the environment can incorporate genetic material without transference from a living vector. Thus, adverse characteristics of the dead organism could be transfered to living organisms capable of reproduction. The probability of this occurrence is generally considered to be low. Nevertheless, the transfer is possible, and may require treatment of sterilized products as biologicals.

7. Are existing regulatory instruments and administrative structures sufficient to protect human health and environment from genetically engineered organisms, and are regulatory interpretations and jurisdictions clear for biotechnology products?

Existing regulatory instruments which could be applied to biotechnology were designed with reference to chemical products. The preceding questions in this section, and many other relevant questions, are not specifically addressed by existing regulatory instruments. The Canadian Environmental Contaminants Act, for example, is specifically limited to consideration of non-biological contaminants, athough this is presently under review.

From Henley's (1986) review of existing Canadian legislation, it is clear that federal and provincial jurisdictions overlap and that inconsistencies occur between jurisdictions. Since the working of existing legislation is often ambiguous with respect to biological products, legal analysis will likely be required to determine the most appropriate

legislation on a case-by-case basis. Similar regulatory confusion in other countries has resulted in establishment of coordinating agencies to advise regulatory agenicies and/or interface with industry in a consistent manner. A Canadian coordinating body may be required to determine the adequacy and most appropriate application of existing legislation, as discussed in greater detail in Section 4.0.

3.0 THE NATURE OF BIOTECHNOLOGY

3.1 Definition of Terms

The term "biotechnology" is a neologism which has not been included in any major dictionary. Most new terms acquire meaning by usage, which tends to stabilize over a period of time. Until usage becomes consistent, particular care must be taken to define all terms used in policy statements, guidelines or regulations, so as to avoid general confusion.

Biotechnology was defined by Harsanyi (1985) in the American Association for the Advancement of Science Seminar Series conducted in 1983 for the U.S. EPA, as "the use of biological systems to transform, concentrate, degrade, or otherwise alter materials for scientific purposes at an industrial level". Canada's National Biotechnology Advisory Committee (1984) defined the term simply as "expansion and application of biological knowledge toward practical ends". The term was not defined by the OECD (1985), the Australian Recombinant DNA Monitoring Committee (1985) or the Canadian Medical Research Council (1977). These last-named groups referred to "recombinant DNA techniques" in their guideline documents, without using the term "biotechnology".

In its broadest sense, then, biotechnology includes the traditional fields of medicine and agriculture. Yet, many traditional practices within these fields are generally considered to be adequately regulated in Canada and other developed nations. It is the newer practices such as the cellular and molecular genetic engineering techniques, which have become the focus of recent attention in the media and the courts. These newer techniques are often implied when the term biotechnology is used in its narrow sense. Genetic engineering includes recombinant DNA and cell/protoplast fusion techniques.

Some traditional aspects of biotechnology are subject to the same general types of concern for public health and environmental protection as are the newer genetic engineering techniques. These include large scale fermentation systems and breeding programs for strain improvement by mutation and artificial selection. Some strain improvement by these traditional methods often follows the development of a recombinant organism by genetic engineering techniques.

The commercial potential of the products of some of the newer biological processes such as rDNA and cell fusion has stimulated the need for more specialized and cost-efficient production technologies. These new engineering technologies allow the commercial production of both genetically engineered micro-organisms and the more specialized mass production of naturally occurring organisms. It is this enabling scale-up engineering technology which poses as great a potential concern as genetically engineered organisms if it is not appropriately applied.

The following Section 3.2 defines and describes both genetic engineering and traditional biotechnology processes which have been subject to recent debate on policy and regulation. Examples of specific applications are given in Section 3.3.

3.2 Processes

The fermentation process has been used for decades. It is defined by Harsanyi (1985) as "a general process whereby micro-organisms produce products in a closed system". This is a broad definition, but probably reflects current usage by industry. Only the fermentation product is intended to leave the closed containment system. Elaborate precautions are taken to keep the micro-organisms confined within the containment system, and to keep other organisms out. These precautions tend to be cost-effective since contamination of the system would reduce or curtail productivity.

The organism used in the fermentation system may be a natural wild-type strain, a naturally occurring mutant strain isolated by artificial selection methods, an induced mutant strain produced by chemical or radiation mutagenesis, or a recombinant organism produced by genetic engineering techniques. The majority of organisms now in use are naturally occurring strains, but the balance may be expected to shift toward induced mutants and recombinant organisms.

As of 1981, more than 200 industrial scale products were being produced by fermentation, including 90 antibiotics, 40 enzymes, about 20 amino acids, some 20 organic acids and solvents, and about 35 vitamins, growth factors and other products. Specific examples, with particular emphasis on products produced by recombinant organisms, are given in Section 3.3.

The mutation/selection process has been widely used in traditional breeding programs for improvement of plant and animal species, particularly crop plants and domestic livestock. Mutations are induced by external radiation or chemical treatment of parental stock to increase variability in the next generation. This is followed by an artificial selection phase in which organisms with desired traits are selectively propogated. The selection process tends to reduce the variability of the selected population, while gradually shifting the average population characteristics in the desired direction over several generations.

Mutagenesis is more often used in plant breeding programs where large numbers of offspring are produced by each plant and, therefore, high levels of dominant lethality induced by high radiation doses can be sustained by the population. Seeds are typically used as a convenient stage for radiation treatment, with acute doses ranging up to 10-100 KR X- or γ-radiation.

A broad spectrum of mutations may be induced by radiation treatment. While spontaneous mutation rates are typically on the order of 10^{-6} to 10^{-5} mutations/locus, mutation rates may be increased by one to three orders of magnitude in plant breeding programs. The majority of the mutations induced are neither phenotypically nor genotypically characterized. Only those characteristics of particular interest to the breeding program are quantified. Some undefined characteristics may be eliminated during the selection phase, while others may persist through successive generations and become incorporated into the genome of the improved plant species.

Thus, traditional breeding methods can induce extensive genetic changes, with limited characterization of the altered genome. The traditional methods differ from cellular and molecular genetic engineering techniques in that they are limited by species boundaries, with the exception of a few closely related species which are able to produce viable hybrids by normal reproductive means. Genetic engineering greatly increases the size of the gene pool from which potentially useful (or deleterious) genetic characteristics can be drawn.

Genetic engineering is defined by Harsanyi (1985) as the "alteration of the hereditary materials of biological systems to improve their performance". This broad definition would include the mutation/selection methods used in traditional plant and animal

breeding programs. Hardy (1985) makes the distinction between "organismal genetic engineering", which includes traditional breeding techniques, "cellular genetic engineering", and "molecular genetic engineering". The cellular and molecular techniques are quite recent, and more powerful than traditional breeding techniques in that they permit genetic material from unrelated organisms to be combined. These newer techniques are often implied when the term genetic engineering is used in its narrow sense.

Cell/protoplast fusion techniques are used in genetic engineering at the cellular level. These techniques involve fusion of two cells, or protoplasts, so that the entire complement of genetic material, from both cells or protoplasts, is confined within a single new hybrid cell or protoplast. Plant and bacterial cells have rigid cell walls which interfere with the fusion process; therefore, the portion of the cell within the cell wall (i.e., the protoplast) is preferred for fusion. The hybrid cell formed by fusion combines many of the characteristics of the parent cells. In plant and animal cells, these characteristics may be encoded either in the chromosomal DNA, within a cell nucleus, or in the extra-chromosomal DNA, which resides outside the nucleus as a plasmid. In bacterial cells, this distinction is less obvious, since there is no clearly delineated cell nucleus; however, there is a large circular DNA molecule in the centre of a bacterial cell which is often defined as a chromosome and distinguished from other DNA in the cell.

While hybrid cells exhibit many of the characteristics of the parent cells, there is no guarantee that any particular parental cell characteristic will be expressed in the hybrid. Interactions between the two parental genomes are difficult to predict, and it is conceivable that unexpected properties may arise. The predictability of the process is increased when the properties and genetic composition of both parental cell lines are well known.

Hybrid plant cells can grow, replicate and differentiate in culture to form a viable multicellular embryo. Some of these embryos may eventually become mature plants, capable of reproduction. Hybrid animal cells can also be grown in culture, and do form multicellular tissues; however, differentiation is less plastic, and viable organisms have not yet been produced from animal somatic tissue culture.

Hybrid animal cells are used in production of monoclonal antibodies for both diagnosis and treatment of animal diseases. This technique, illustrated in Figure 3.1, involves fusion of a blood lymphocyte producing a specific antibody, with a myeloma tumor cell capable of rapid proliferation. The result is a rapidly proliferating cell culture that produces a specific antibody. Antibodies are blood proteins which attack specific foreign substances in the body, such as disease organisms.

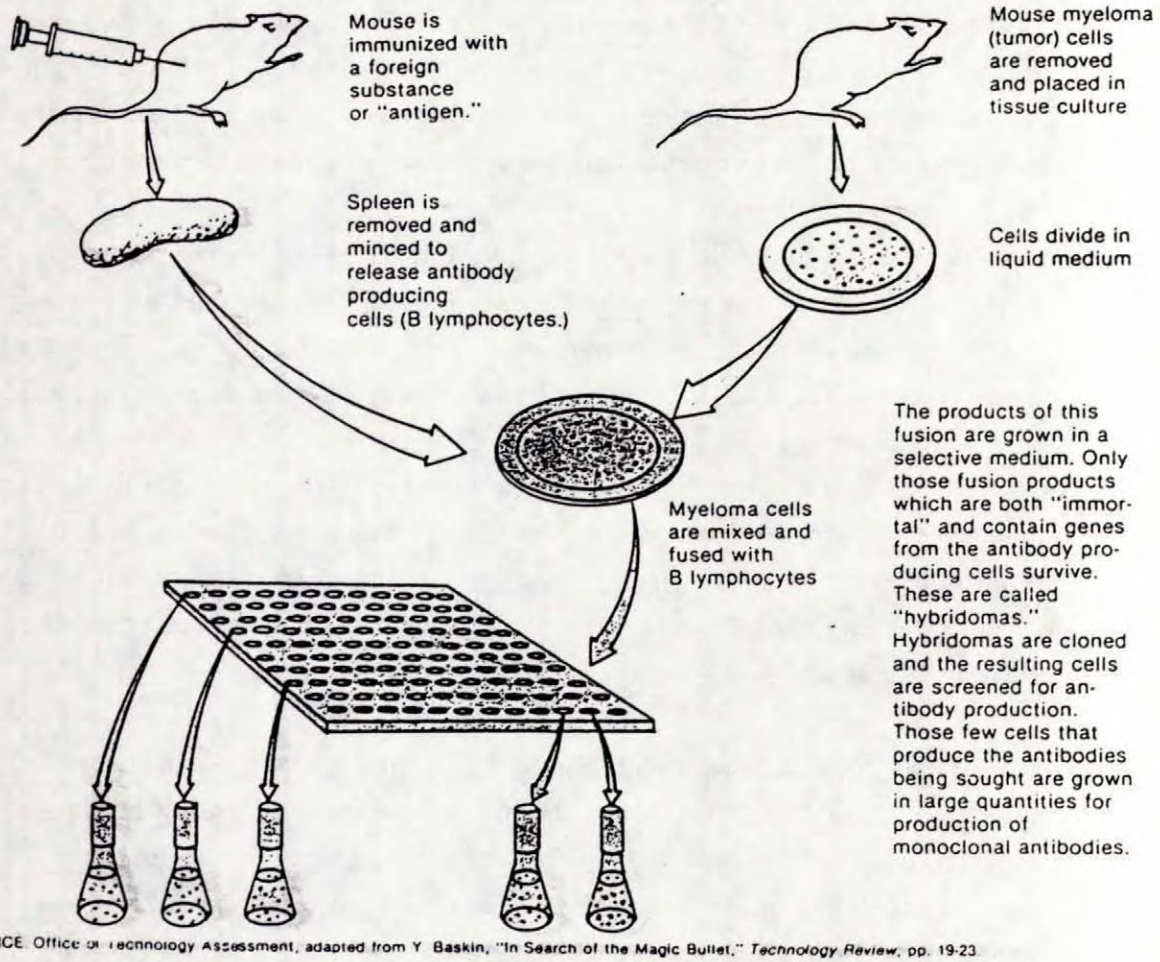
Recombinant DNA techniques are used in genetic engineering at the molecular level. These techniques involve precise excision of specific DNA segments from one organism, or synthesis of new DNA segments, and transfer of those segments into the DNA of another organism, using restriction enzymes to break the DNA molecules at specific points, and plasmid vectors to accomplish the transfer (Figure 3.2). Restriction enzymes are also involved in the normal cellular processes of genetic replication and repair.

The Canadian Medical Research Council (MRC, 1977) defines recombinant DNA as a "DNA molecule that is composed of two or more molecules that were physically separate prior to recombination". The international Organization for Economic Cooperation and Development (OECD, 1985) defines recombinant DNA molecules more specifically as "either (i) molecules which are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) DNA molecules that result from the replication of those described in (i) above". The latter definition explicitly recognizes that the offspring of a recombinant DNA organism contains recombinant DNA. Both definitions include genetic recombination within or between species.

Application of recombinant DNA methods requires some knowledge of the genetic makeup of both the DNA donor and the host organism. Usually, the objective is to transfer a specific gene into the host, and the structure and function of the gene, at least, has been characterized. The DNA segment which carries this gene is defined by its endpoints, and the specific restriction enzyme used to break the DNA molecule at those particular points. Often, many of the other genes on the donor segment have been characterized, and an insertion point in the host genome has been identified.

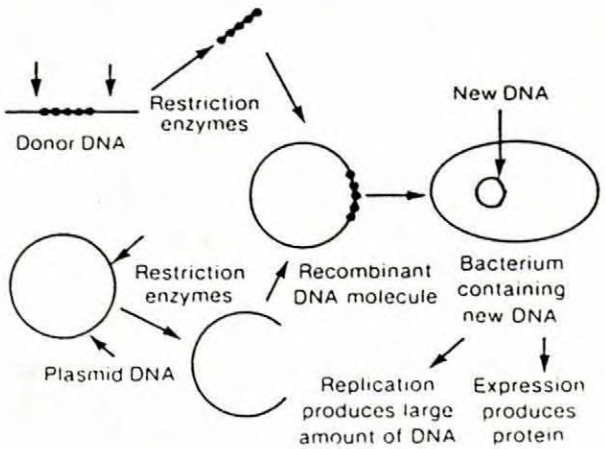
The necessary precision of recombinant DNA technology lends itself to small, specific and well-defined alterations in the genetic system of the host organism. Thus, while the

FIGURE 3.1: MONOCLONAL ANTIBODY PREPARATION (OTA, 1984)



SOURCE: Office of Technology Assessment, adapted from Y. Baskin, "In Search of the Magic Bullet," *Technology Review*, pp. 19-23.

FIGURE 3.2: RECOMBINANT DNA TECHNIQUE (OTA, 1984)



Restriction enzymes recognize certain sites along the DNA and can chemically cut the DNA at those sites. This makes it possible to remove selected genes from donor DNA molecules and insert them into plasmid DNA molecules to form the recombinant DNA. This recombinant DNA can then be cloned in its bacterial host and large amounts of a desired protein can be produced.

SOURCE Office of Technology Assessment

potential donor gene pool is vast, there may be less potential for inadvertent genetic change than with other biotechnologies, including conventional mutation/selection methods. This must be balanced against the lack of historical experience with the newer technologies in estimating the risks involved. Methodology for risk assessment will be an issue in implementation of a biotechnology policy (Section 2.1.3).

3.3 Applications

The processes described above and collectively referred to as biotechnology are currently applied in many different industries. Since few of the firms involved rely solely on biotechnology, it is perhaps premature to consider biotechnology as an industry per se. However, the role of biotechnology in the chemical, pharmaceutical, agricultural, food processing and resource recovery industries is clearly expanding. Some products have already reached the marketplace, and many more are under development. Anticipation of future applications of biotechnology will be instrumental in development of an appropriate regulatory approach.

Success in the marketplace ultimately depends on the economics of the process, including costs for raw materials and energy, labour, waste disposal, and product approval. The approval process for pharmaceuticals and food products, in particular, can be prohibitively expensive. Uncertainties in the approval process thus lead to financial uncertainties, and can discourage research and development at an early stage.

Applications of fermentation in industrial chemistry include production of commodity chemicals and fuels (Cooney, 1983) and specialty chemicals (Genex Corp., 1983). Ethanol is an example of a commodity chemical produced by commercial fermentation on a large scale (Skotniki et al., 1982). This process forms the basis of the beer, wine and distillation industries. Organic acids produced on a large scale by fermentation include acetic acid (vinegar) and citric acid, both extremely important products in food processing (Daniel and Whistler, 1981). Other organic acids which could potentially be produced by micro-organisms on a large scale include propanoic acid, fumaric acid and edipic acid (Daniel and Whistler, 1981), lactic acid and levulinic acid (Lipinsky, 1981) and succinic acid (Ng et al., 1983). Fuels, such as isopropanol and butanol, and solvents such as acetone, may also be produced by microbial fermentation (Daniel and Whistler, 1981; Lipinsky, 1981). Elemental sulphur might be produced in quantity by H_2S metabolizing organisms (Haber et al., 1983).

Table 3.1 lists some potentially important commercial chemicals and microbial species which produce them. Many commodity chemicals are now produced as petrochemical products. The shift from petrochemical processing to bioprocessing will likely be slow because of the large infrastructure involved (OTA, 1984). However, research toward that shift is in progress. Increasing the efficiency of microbial processing by genetic manipulation will be critical to that shift. For example, research on improvement of microbial amylases for starch hydrolysis (OTAF, 1982), and improved microbial degradation of ligno-cellulose (Bungay, 1981) and cellulose (Bungay, 1981; Emert and Katzen, 1981) is underway. The National Research Council of Canada has succeeded in transferring cellulase genes into more productive bacteria using recombinant DNA techniques (Montenecant, 1983).

Specialty chemicals are produced in smaller quantities and sold at higher prices than commodity chemicals. Many amino acids used in food processing are considered specialty chemicals (OTA, 1984). For example, glutamic acid, used to make monosodium glutamate, is produced by the bacterium Corynebacterium. Other important amino acids produced by microbial fermentation include methionine, lysine (also produced by Corynebacterium), tryptophan, aspartic acid and phenylalanine. Phenylalanine is used with aspartic acid to produce aspertame. Recombinant DNA research toward improved strains of Corynebacterium is in progress (Genex Corp., 1983) and E. coli mutants which overproduce phenylalanine have been isolated (Choi and Tribe, 1982).

Enzymes produced by microbial fermentation for industrial application include alkaline protease, used in detergents; alpha-amylase, used in starch liquefaction; glucoamylase, used in glucose production from starch; glucose isomerase, used in glucose-fructose interconversion for synthesis of sweeteners; rennet, used in cheese manufacturing; and pectinases, used in juice clarification. The micro-organisms used in fermentation of some of the more important industrial metabolites and enzymes are listed in Table 3.2. These products are typically produced in highly contained systems, not so much for protection of the public and environment, as for protection of an often delicate fermentation system from outside contamination. Thus, there is a profit incentive in good safety practice.

Recombinant DNA techniques are expected to facilitate expansion of the enzyme industry (Eveleigh, 1981). For example, the gene for rennin, the calf-milk-clotting

TABLE 3.1: SOME COMMODITY CHEMICALS AND MICROBIOLOGICAL SOURCES

Chemical	Microbiological Source
Alcohols:	
Ethanol	*Zymomonas mobilis*
	Saccharomyces sp.
Organic Acids:	
Acetic Acid	*Acetobacter* sp.
	Clostridium aceticum
Citric Acid	*Aspergillus niger*
Lactic Acid	*Rhizopus oryzae*
	Lactobacillus bulgaricus
Fumaric Acid	*Rhizopus nigricans*
Gluconic Acid	*Aspergillus niger*
Propionic Acid	*Propanio-bacterium* sp.
Solvents:	
Acetone-butanol	*Clostridium acetobutylicum*
2,3-Butanediol	*Bacillus polymyxa*

TABLE 3.2: SOME SPECIALTY CHEMICALS AND MICROBIOLOGICAL SOURCES[1]

Chemical	Microbiological Source	Application	Trade Name
Enzymes			
Alkaline protease	*B. subtilus*	detergents	Alkalase
Alpha-amylase	*B. licheniformis*	starch liquefaction	Termamyl
Glucoamylase	*Aspergillus niger*	starch-to-glucose conversion	AMG
Glucose isomerase	*Bacillus* sp.	glucose-fractose interconversion	Sweetzyme
Rennin	rDNA *E. coli*	cheese manufacturing	-
Rennet	*Mucor miehei*	cheese manufacturing	Renilase
Amylases	*Bacillus* sp.	various uses	BAN, Aquazym
Pectinases	*Aspergillus niger*	juice clarification	Pectinex
Amino Acids			
L-Citrulline	*Bacillus subtilis*	-	-
L-Lysine	*C. glutamicum*	-	-
L-Ornithine	*C. glutamicum*	-	-
L-Phenylalinine	*Arthrobacter paraffineas*	-	-
L-Threonine	*Escherichia coli*	-	-
L-Tyrosine	*Corynebacterium* sp.	-	-
L-Valine	*C. glutamicum*	-	-

[1] From King (1985).

enzyme used in making cheese, has recently been transferred to the bacterium E. coli and successfully cloned (Harris et al., 1982). An enzyme product produced by microbial fermentation will soon be on the market (Genex Corp., 1983).

Vitamins produced by microbial fermentation include vitamin B2 (riboflavin) and B12 (Florent and Ninet, 1979). Recently, a high-yield strain of Bacillus subtilis for improved production of vitamin B2 has been developed using recombinant DNA techniques (Enei et al., 1975).

Single cell protein (SCP) is a microbial product which may be used increasingly in domestic animal feeds. The product consists of high-protein microbial cells harvested in bulk. Large-scale fermenting systems have recently been developed to produce SCP (Waterworth, 1981), assisted by recombinant DNA research on improved strains which are more easily harvested (Litchfield, 1983).

Other specialty chemicals include fatty acids, which are widely used in cosmetics, plastics, greases, emulsifiers, cleaners, paints, foods and flotation reagents; fatty alcohols, used to make palsticizers, microbial oils, biosurfactants, steroids, aromatics and biopolymers (OTA, 1984). Biopolymers have great potential as microbial products, but genetic manipulation in strain improvement may be difficult as biopolymer production is usually a polygenic trait.

Biopolymers are polysaccharides used as thickeners and floculants. Bacterial biopolymers have many uses. Products such as xanthan gums, produced by Xanthomonas campestris; gellan, produced by Pseudomonas sp. (Wells, 1977) and alginates, produced by Azobacter vinelandii (Jarman, 1979) have been widely used in food processing for many years. Pullulan, produced by Aureobasidium pullulans (Jeans, 1977) is a more recent product used in making biodegradable plastics. Emulsan is a biopolymer produced by a recently developed microbial strain which emulsifies oil and may have important environmental applications (Goldman et al., 1982).

Emulsans, like other biopolymers, are currently produced by fermentation in contained systems. Emulsan products have been field tested and marketed. They may be used in resource recovery and waste treatment. Residual oil recovery, for example, requires creation of sufficient underground pressure to force residual oil out of its underground

reservoir or matrix. This has been accomplished mechanically by pumping dense liquids into the reservoir. However, new techniques under development would involve injection of emulsan-producing bacteria, or other bacteria capable of generating gaseous pressure directly into the reservoir. Some degree of genetic modification would likely be required to permit the organism's survival under the high temperature and pressure conditions of the reservoir. Thus, successful development of this technology may lead to intentional environmental release of genetically engineered organisms.

Emulsans may be used in waste treatment for degradation and cleanup of waste oil. These products spread over the surface of an oil spill, rapidly dispersing it and permitting normal environmental hydrolytic processes to degrade the waste. A variation on this process involves treatment of the waste oil with the living emulsan-producing micro-organism. This actually enhances the degradation process, and provides a continuing supply of emulsan, but raises new concerns about environmental impacts of released micro-organisms. The micro-organisms now used occur naturally, although the potential exists for development of genetically modified strains.

Other waste treatment applications of biotechnology include sewage treatment, where micro-organisms are used in floculation, dewatering, degreasing, and removal of toxic metals, organics and slimes. Improvements on these processes may involve genetic manipulation by recombinant DNA or other techniques. For example, a new strain of Pseudomonas has recently been developed which is capable of degrading the herbicide 2,4,5-T (Kilbane et al., 1982). Mouse genes for metalothionine have been successfully transferred to bacteria and cloned (Mbikay et al., 1983; Palmiter et al., 1982). Metalothionines are proteins responsible for sequestration of metals. Thus, the recombinant bacteria would act to remove metals from sewage if grown in that medium. Development of cold tolerant sewage bacteria for northern sewage treatment systems is needed, and would be a likely objective of Canadian biotechnology research.

Bacterial leaching is currently used in mineral recovery from low grade ore (Brierly, 1982; Volger, 1981). Ten percent of U.S. copper is now recovered by bacterial leaching, and some Canadian uranium mines have relied entirely on this process in the past (Brierly, 1978). The bacterium, Thiobacillus ferrooxidans, dissolves the mineral from the ore, accumulating it externally. The degree of containment varies, but is usually minimal. In one version of the process, an innoculated slag heap is flooded with water

(Figure 3.3). A version which has been used in Canada involves flooding the mine. The bacterium presently used is a naturally-occurring organism which has not been genetically manipulated. However, genetically modified superior strains may well be developed in future (Brierly, 1982; Chakrabarty, 1978; Ralph, 1982).

Pharmaceuticals which may be produced by fermentation or cell culture include antibiotics, such as penicillin and streptomycin; blood products, such as human serum albumin; hormones, such as human growth factor, and insulin; vaccines and diagnostic products.

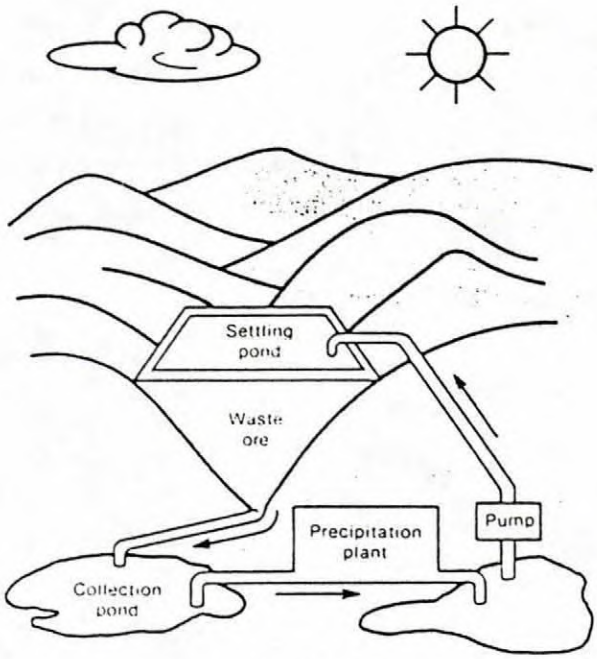
Antibiotics are produced naturally by micro-organisms as ecological defense mechanisms against competitors. Penicillin, for example, produced by a mold, inhibits the correct formation of the mucopeptide component of bacterial cell walls, thus preventing multiplication of bacterial competitors. Streptomycin, aureomycin, terramycin and neomycin are produced by Actinomycete bacteria for inhibition of other bacteria. Their antibacterial action is useful to man in treatment of bacterial pathogens.

Radiation mutagenesis and selection of high-yield mutant strains have been used for many years to improve antibiotic yields (Vournakis and Elander, 1983). Recombinant DNA techniques remove the random element from the strain-improvement process. However, even the improved strains are still producing natural products, and the product rather than the organism is marketed.

Monoclonal antibodies offer great potential as pharmaceutical products. Antibodies, produced by blood lymphocytes, are proteins which bind specificaly to particular disease organisms or foreign substances in the body, thereby inactivating them. Lymphocytes responsible for producing particular antibodies can be grown in tissue culture, and the antibodies which they produce can be recovered for later use in immunotherapy. This is essentially a fermentation technique.

Antibody production can be greatly enhanced by using cell fusion methods to combine lymphocytes and tumor cells. The resulting cell culture, or hybridoma, has the rapid growth characteristics of a tumor, and produces the desired antibody at an enhanced rate. The basic methods of monoclonal antibody production are illustrated in Figure 3.1, and described in (Gatz et al., 1983; Langone, 1983; Milstein, 1980; Yelton and Scharff,

FIGURE 3.3: BACTERIAL LEACHING OF LOW GRADE ORE (OTA, 1984)



SOURCE: Office of Technology Assessment

1980). Variations in the technique which may prove valuable during scale-up to increase the antibody yield and reduce costs include immobilization of the hybridoma cells (Tsung et al., 1980) and microencapsulation of the hybridoma.

Monoclonal antibodies may be used in therapy, diagnosis and purification of other pharmaceuticals. In therapy, it may be possible to combine specific antibodies with potent drugs which would help to combat localized diseases within the body. The antibody would thus serve as a molecular carrier, attaching the drug to the diseased site, and confining the drug to that location. For example, cytotoxic agents attached to tumor-specific antibodies might be used in chemotherapy for cancer patients, with highly localized cytotoxicity destroying the target tumor but not the surrounding tissues. Such techniques are still under development (Bernstein, 1982; Gilliland et al., 1980; Krolic et al., 1980; Vogel and Muller-Eberhard, 1981) but, if developed successfully, would greatly increase the demand for monoclonal antibodies.

In diagnosis, monoclonal antibodies are used to carry a detectable marker to sites of specific infection, or disease, within the body, or in body fluid samples (McMichael and Faber, 1982; Nowinski et al., 1983). For example, monoclonal antibody tests may be used to diagnose cancer, meningitis, hepatitis B (Wands et al., 1981), Streptococcus, Monococcus, Chlamydia, sickle-cell anemia (Orkin et al., 1982), B thalassemia, or the malarial plasmodium (Cochrane et al., 1982). Early diagnosis of normal conditions, such as pregnancy, can also be accomplished using monoclonal antibodies specific to certain body products, such as human chorionic gonadotropin, a hormone produced during pregnancy.

In pharmaceutical purification, monoclonal antibodies which bind specifically to the pharmaceutical product can be developed. In many cases, it is easier to recover the pharmaceutical-antibody complex from the production stream than the original pharmaceutical product. The antibody is then decoupled from the pharmaceutical to give a highly purified product.

Human hormones, like antibodies but unlike antibiotics, are not naturally produced by micro-organisms. They are often produced in extremely small quantities in the human body, making recovery from donated human organs impossible on a commercial scale. Some hormones, such as insulin, can be recovered from the organs of other animals.

Porcine insulin, for example, is effective in treatment of human diabetes caused by lack of insulin. However, it has undesireable side effects. Other hormones, such as human growth hormone, can be obtained only from human cells. In both cases, recombinant DNA techniques can be used to transfer the gene for the human hormone from a human cell into a bacterium. Fermentation techniques are then used to produce the bacterium, and the hormone, in large quantity.

Human insulin is one of the few hormones yet to be produced by such methods on a large scale. The initial recombinant strain of E. coli produced insulin with a low yield of less than 3% of soluble protein (Cooper, 1985). Improved strains now yield 15% to 30% insulin. The strain improvement process may include traditional mutation and/or selection methods.

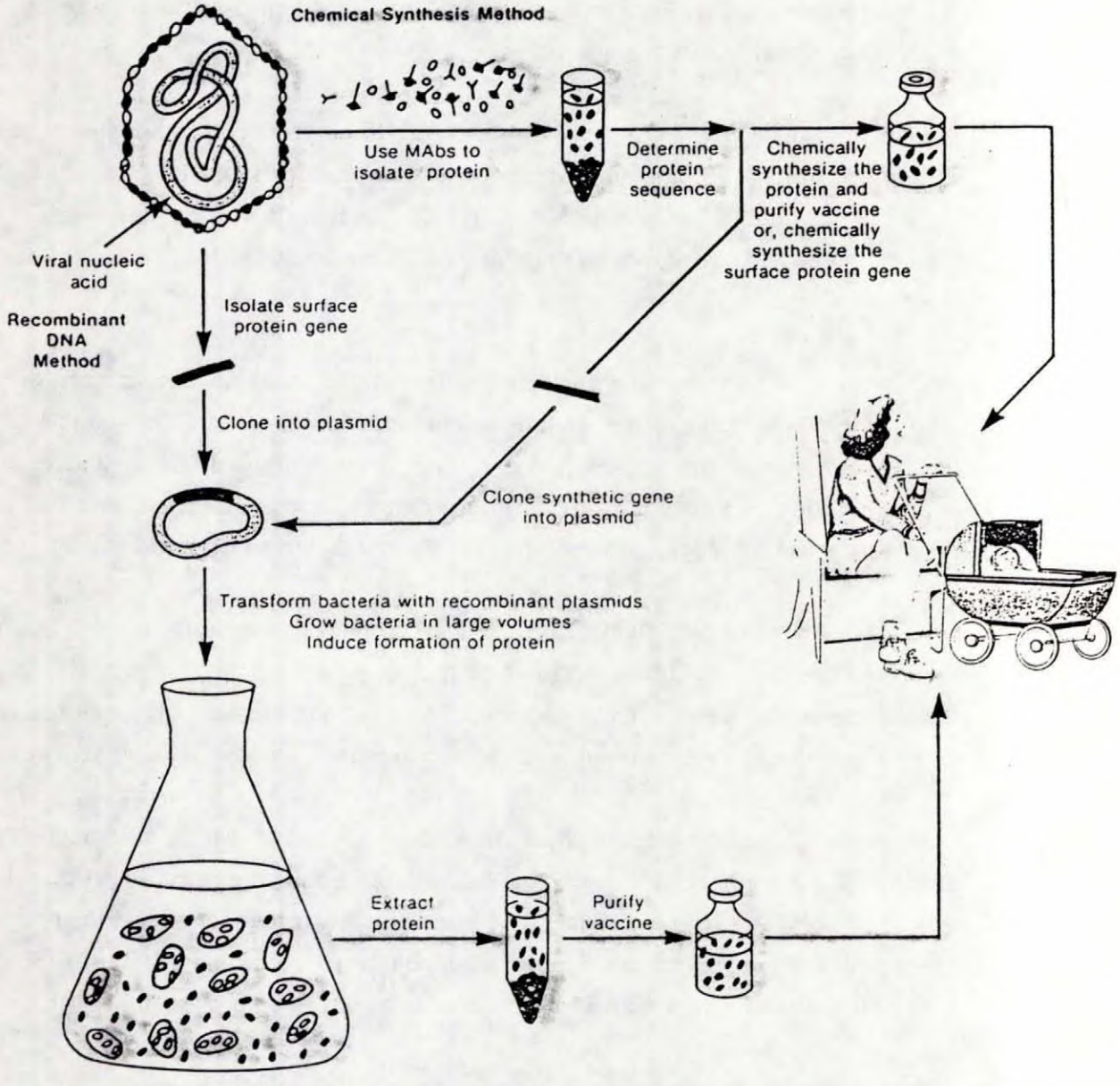
Interferon is a hormone currently under investigation as an anti-cancer agent (Billiau, 1981). Interferon from other animal species cannot be used in humans, and extraction from either human or other animal tissues is extremely difficult and expensive. Its recent production in quantity by fermentation of recombinant bacteria has permitted a recent increase in cancer research related to interferon treatment.

Other human hormones which have been cloned in bacteria include human growth hormone, which acts to regulate growth, somatostatin and somatomedin. Research toward cloning of neuroactive peptides, such as endorphins and lymphokinases, which are involved in lymphocyte communications and immune response is currently in progress.

Non-hormonal blood products which have been cloned in bacteria include human serum albumin (Low et al., 1981) and clotting or antihemophilic factors (Bloom, 1983; Chou et al., 1982). The latter are important in treatment of hemophelia. Fibrinolytic enzymes which are involved in the breakdown of blood clots would also be useful products if the genes for these enzymes could be cloned and the enzymes produced in quantity.

Vaccines can be developed by using recombinant DNA techniques to eliminate genes for infectious properties from disease organisms or, alternatively, to remove genes for the antigens which ellicit the immune response and clone them in a non-infectious species. Any non-infectious product which bears the antigen can be used to confer immunity. The process of developing vaccines is illustrated in Figure 3.4. Recombinant DNA vaccines

FIGURE 3.4: RECOMBINANT DNA VACCINE PREPARATION (OTA, 1984)



In the **chemical synthesis method**, proteins that comprise the viral surface are isolated, often with the use of monoclonal antibodies. The protein sequence is then determined. Based on the sequencing information, large amounts of the protein or portions of the protein are made chemically for use as the vaccine; alternatively, the sequencing information may allow chemical synthesis of the gene that encodes the protein (or a small portion of the protein). This synthetic gene is cloned via rDNA techniques.

In the **recombinant DNA method**, the gene that encodes the viral surface protein is isolated and cloned into an appropriate vector (such as plasmid), transformed into a host (such as a bacterium or yeast), and the host is grown in large quantities. Formation of the protein by the rDNA and isolation of the protein results in the subunit vaccine.

SOURCE: Office of Technology Assessment

are under development for viral diseases, such as polio, influenza, herpes, rabies and hepatitis, bacterial diseases such as Streptococcus and Gonococcus, and parasitic diseases such as malaria (Newmark, 1983).

Agricultural applications of biotechnology, in the broad sense, include the traditional applications of biological pest control and plant breeding by mutation and selection, as well as the more recent protoplast fusion and recombinant DNA applications. Actual and potential applications are reviewed by OTA (1981), Barton and Brill (1983) and Day (1983). A well known example of biological pest control is the widespread use of the bacterium Bacillus thuringiensis to control lepidopteran insect pests, such as Plodia interpunctella (the gypsy moth). Plodia feeds on stored grain and field crops. The bacterium attacks the insect producing several poisonous exotoxins (B-exotoxin and α-exotoxin). The pest control product (BT) includes both bacterial spores and exotoxins, produced in quantity by fermentation. Thus, the bacteria grown continue to produce the toxins in fields and seed bins which have been inocculated.

Biological pest control has several advantages over chemical pest control methods. It is directed specifically toward the target pest species, thereby limiting potential adverse effects on other plants and animals; and the control organism has the potential to co-evolve with the insect pest, making it more difficult for the pest to acquire permanent resistance to the control agent through natural selection in the field. However, resistance can develop with prolonged use of the control agent, as indicated by recent USDA research on BT resistance (McGaughey, 1985).

Bacillus thuringienses occurs naturally in insect hosts but does not persist well in the field following application. Recently, Monsanto Agricultural Products has succeeded in incorporating the gene for endotoxin production into another bacterium, Pseudomonas fluorescens, using recombinant DNA techniques (Dixon, 1985). The host organism is better adapted to agricultural environments, since it tends to colonize plant root systems. Thus, the recombinant organism could provide long-term protection against soil-borne pests following a single application. This application would constitute intentional release of a recombinant organism designed to persist in the environment.

Nitrogen fixation has been the focus of a great deal of agricultural research using recombinant DNA techniques. Nitrogen is an essential nutrient for agricultural

production of plant protein. It is supplied to the soil by fertilization in order to replace that lost each year with the harvest. Leguminous plants, such as beans and peas, support a nitrogen-fixing bacterium, Rhizobium sp., in a system of root nodules. The bacterium fixes nitrogen from the atmosphere in a form usable by the plant, permitting plant growth in nitrogen-poor soils, and reducing fertilizer requirements. Nodule formation can be encouraged and nitrogen supply enhanced by inocculation of legume seeds with the bacterium, produced in quantity by fermentation.

Nitrogen-fixing bacteria in root nodules must compete, however, with other rhizobia (root-dwelling micro-organisms). Recombinant DNA techniques may offer the possibility of highly competitive, and therefore more effective strains. Improved Rhizobium strains with some promise have already been developed (OTA, 1984). It may even be possible to transfer the genes for nitrogen-fixation into more competitive micro-organisms, or into the plant itself, or to transfer genes for nodule formation into non-leguminous crop plants. The gene for leghemoglobin, which maintains the oxygen deficient nodule environment essential to nitrogen fixation, has now been identified and transferred from the host plant to a bacterium (Sullivan et al., 1981), but many more genes are likely involved in the complex process of nodule formation.

Recombinant DNA techniques may also be used to improve crop plants with respect to herbicide resistance, cold or drought tolerance, growth rate and yield. Chemical weed control is much easier with a crop plant which tolerates the herbicide. Seedlings are particularly sensitive to herbicides at a point in their life history when weed control is critical. Alternatively, growth characteristics permitting successful competition with weeds, or other properties of pest or disease resistance, may be transferred directly to the crop plants, reducing the need for pest control. Resistance factors tend to be simple genetic traits, often carried on bacterial plasmids, and therefore quite amenable to genetic manipulation and transfer to plant cells (Leemans et al., 1981).

Cold and drought tolerance, if successfully incorporated into crop plants, could greatly increase the amount of arable land available for food production, and the length of the growing season in seasonal climates. Some progress has been made in development of hardy varieties through traditional plant breeding methods. However, recombinant DNA techniques promise even greater progress.

A novel approach to increasing plant growing seasons involves the use of the ice-minus bacterium (Pseudomonas syringae). The wild strain secretes a protein which provides a nucleus for initiation of ice crystal formation in plant tissues (and also for rain droplet formation in the atmosphere). Advanced Genetic Sciences succeeded in deleting the gene responsible for ice nucleation, using recombinant DNA methods, to produce the ice-minus mutant strain. The mutant may prevent frost damage if applied to a plant crop in sufficient quantities to supplant the wild strain (Miller, 1983). Field trials of the technique have been planned, and approved by the U.S. Environmental Protection Agency (Sun, 1985). However, local opposition has so far prevented field trials from going forward (Sun, 1986b). A public concern is that the ice-minus mutant may decrease local rainfall.

The ice-minus case is interesting in that the mutant organism does not really qualify as a genetic recombinant according to the OECD or MRC definitions. No foreign genetic material has been added to the host organism, and there is no logical reason to consider it any differently than many other mutant organisms which have been released to the environment through plant breeding programs. In fact, the mutations in this case are extremely well-defined, unlike many which may be produced in traditional plant breeding.

Growth and yield enhancement through genetic manipulation may also help to increase agricultural food production. Both plant and animal breeding programs currently use mutation/selection methods to achieve this goal. Plant breeding objectives include rapid growth and/or maturation, so that more crops can be produced each season, enhanced seed production, and increased oil content of seeds used for vegetable oil extraction. Animal breeding objectives include rapid weight gain in poultry and beef cattle, and increased milk production in dairy cattle.

Recently, the U.S. Department of Agriculture has succeeded in transferring foreign growth hormone genes into mice, by injection of the foreign genetic material into fertilized mouse eggs. Human growth hormone genes are preferred because of their ready availability. The recombinant eggs are then reimplanted into surrogate mothers for normal gestation and birth. Recombinant mice grow more rapidly and attain a larger size than normal. The next step in the gene transfer program is to develop this technique for livestock such as sheep and pigs. A lawsuit filed in October 1984 and still before the

U.S. courts was intended to prevent the USDA from pursuing this line of research (Fox, 1984). Objections focussed on potential environmental disruption by genetically modified livestock, uncertain economic benefits, and the ethics of crossing mammalian species barriers.

Animal husbandry applications of biotechnology are similar to human health applications (Zimmer, 1982). Feed additives produced by microbial fermentation are widely used as growth promoters (OTA, 1979) and antiparasitics (Campbell, 1983). Growth hormones have been produced by fermentation and field trials of these products have taken place (Peel et al., 1981; Zimmer, 1982). Monoclonal antibodies have been used to treat bacterial scours (Zimmer, 1982) and to provide specific immunity against rabies (Melchers et al., 1978). Monoclonal antibodies are now used in diagnosis of animal diseases, such as blue-tongue and equine infectious anemia, and many other monoclonal diagnosis tests are under development, including tests for canine parvovirus, canine rotavirus, feline leukemia virus and canine heartworm (OTA, 1984).

Vaccines have been developed for bacterial scours using recombinant DNA techniques to clone non-infectious antigenic components of the disease organism. Other recombinant DNA vaccines are under development. Genes for non-infectious components of fowl plague, influenza, vesicular stomatitis, herpes simplex virus and rabies have all been isolated and cloned. A recombinant DNA vaccine for foot and mouth disease, developed by Genentech and the U.S.D.A. (Bachrach, 1982; Della-Porta, 1983), was recently field tested, but results were not encouraging (Kleid et al., 1981).

3.4 Risk Factors

The nature and degree of potential risk involved in the practice of biotechnology depends upon the particular product, process and application under consideration, and the specific details of each case. Many potential risks have been suggested, and there is often considerable disagreement among experts on their relative magnitude and importance. It would be prudent, at this early stage in the science of biotechnology risk assessment, to consider a broad spectrum of potential risks during the evaluation of each case. Nevertheless, a conceptual framework based on general categories of product, process and application may help to structure and standardize risk assessment procedures, with emphasis on the areas of most likely hazard.

The conceptual framework proposed by the U.S. EPA for consideration of potential risk emphasizes the biotechnology process by which a product is derived, as well as the application (EPA, 1984). The rationale for this approach is that it facilitates designation of "new" products within the agency's jurisdiction by defining them as the result of particular novel processes. The implication is that products of certain processes, such as recombinant DNA techniques or cell fusion, should be considered new and subject to risk assessment, whether or not they exist in nature or have been previously approved as conventional products. Similarly, the U.S. FDA (1984) proposes to consider drugs and food additives produced by recombinant DNA techniques as new products subject to approval, even if they are identical to previously approved products; however, products and associated risk assessment considerations are categorized by application (human vs. animal).

The Australian RDMC (1985) confines itself to consideration of recombinant DNA processes, specifically excluding other techniques such as cell fusion, and emphasizes the product application as a framework for risk assessment. The OECD (1985) also considers risk factors associated with recombinant DNA techniques according to the application (human vs. environmental or agricultural).

Regardless of the conceptual framework utilized to structure risk assessment procedures, common risk factors emerge. These are discussed below within a biological framework although, for regulatory purposes, a process or product-oriented approach may be more convenient.

3.4.1 Pathogenicity

Microbial strains which are intended to be pathogenic to agricultural pests are likely to be considered for development as commercial biotechnology products (OTA, 1984). Naturally-occurring bacteria in this category include Bacillus thuringiensis (see Section 3.3). Other known pathogens are listed in Bergy's Manual (Buchanan and Gibbons, 1974). Unintended pathogenicity in recombinant organisms must also be considered as a potential risk. Pathogenicity is of primary concern whenever living organisms are released to the environment, either in a biological product, or as contaminants of ostensibly non-living products, or as waste products from an otherwise contained fermentation system.

When pathogenic traits are known to exist in an organism considered for release, the host range of the pathogen must be clearly identified. Pathogens with a broad host range, or with hosts closely related to man should be considered to represent a greater potential risk than pathogens which are only capable of infecting their intended pest target.

Infectivity tests on a broad range of potential host organisms should be performed. These tests should be designed to quantify the virulence of the infective agent, and identify all vector organisms capable of transmitting the agent to a susceptible host. Guidelines for pathogenicity testing are included in the EPA's Pesticide Assessment Guidelines (EPA, 1982). Highly infective and virulent pathogens of a particular host represent a greater potential risk than weaker infective agents.

Unexpected pathogenic traits can potentially arise in genetically engineered organisms. These may arise either as cryptic genes, not expressed in a DNA donor but expressed in the recipient organism, or as mutations which arise in the recipient following environmental release. The likelihood of such an unexpected event occurring can be assessed to some extent by consideration of the evolutionary relationships of donor and recipient organisms. Organisms with known pathogenic relatives are more likely to contain some of the genes contributing to pathogenicity than are organisms without pathogenic relations.

Unfortunately, evolutionary relationships of micro-organisms are poorly understood (Stackebrandt and Woese, 1984). This is due in part to the tremendous genetic plasticity of micro-organisms and their general lack of genetic isolation (Sonea and Panisset, 1980). Genetic isolation of one species from another is a cornerstone of our species concept. A workable species concept is required, not only for interpretation of evolutionary relationships, but also for retrieval of information concerning a particular organism. A tentative representation of evolutionary relationships among bacteria is suggested by Strauss et al. (1985).

The likelihood of a mutation occurring which would convert a non-pathogenic organism to a pathogen depends on the degree of mutational insult, as well as evolutionary relationships to other pathogens. Brill (1985) points out that pathogenicity is a complex trait, which would require appropriate simultaneous mutations at multiple genetic loci in order to be induced in an organism without pathogenic relatives. However, undirected

mutagenesis, as part of a breeding program, may increase the probability of such simultaneous events to a level requiring serious consideration of the risk (Alexander, 1981; Colwell et al., 1985).

The likelihood of a cryptic gene for pathogenicity being transferred to a recipient organism depends on the amount of genetic material transferred and the degree to which it has been characterized.

Large quantities of donor DNA increase the potential risk. Large quantities are routinely transferred by cell/protoplast fusion methods. Recombinant DNA methods using restriction enzymes usually transfer much smaller quantities and lend themseves more readily to characterization of the donor material. Requirements for complete characterization of foreign DNA and delimitation of the functional ends of the desired DNA sequence would minimize the chance of accidental transfer of pathogenic or other hazardous traits.

When living organisms are not required for efficacy of the biotechnology product, the risk of pathogenicity can be eliminated by complete sterilization of the product prior to use. Complete sterilization may be difficult to achieve due to the logarithmic nature of dose-survival relationships (Harrison and Hattes, 1985), and difficult to verify. However, sterilization to the point at which no living organisms can be detected will minimize the risk of releasing pathogenic or other hazardous organisms. Sterilization methods include the use of heat and pressure (autoclaving), chemicals (e.g., chlorine) or massive radiation doses. The choice of technique must depend on the viability of the organism and the susceptibility of the product to damage during sterilization.

3.4.2 Toxicity

Toxic products include both non-living chemicals and living organisms that produce toxic metabolites. Many pathogens exert their effect by producing toxic metabolites within the body of the host. However, non-infective organisms may also produce toxins.

When living organisms are included in the toxic product, many of the risk factors which apply to pathogens require consideration. In particular, the amount and degree of characterization of foreign DNA in the organism, and its evolutionary relationship to

other toxin-producing (or pathogenic) organisms should be considered. Additional considerations apply to the toxin itself. These are discussed below.

Toxic effects of the product should be determined in a wide variety of organisms. Various types of toxic effect are possible, and appropriate test protocols vary with the test organism and the effect being measured. Highly toxic products, and products which adversely affect a wide variety of organisms, represent a greater potential risk to the environment than toxic products with less potency and more restricted effects. Toxic effects in mammals are generally considered to represent a significant risk to human health. However, mammals are not always convenient test organisms. There is growing recognition that multi-organism testing is the best way to protect both the environment and human health from potential toxic effects.

Acute lethal effects are the most obvious toxic manifestations. These are quantifed in terms of an LC50 concentration of the product or an LD50 dose of the product (ambient concentration or administered dose required to kill 50% of the test organisms within a specified period of time). Acute lethal test durations range from one to four days, depending on the test organism and the protocol followed. Some published protocols include EPA (1982), EPS (1982), MOE (1983), ASTM (1980), OECD (1981) and APHA (1975). Products with low LC50 or LD50 values represent a high potential risk, within the medium or by the administration route on which those values are based.

Chronic lethality is also used as a standard toxicity measure, expressed as an LC50 or LD50 over a much longer test duration. Chronic lethality is usually observed at lower concentrations and doses than acute lethality, and is thus a more sensitive measure of toxicity. Published test protocols include EPA (1982) and OECD (1981). Often these tests are reserved for a second tier of toxicity testing, to be used only with products which pass guidelines for less rigorous tests.

Reproductive performance of a test organism exposed to a product over a long period is also used to assess potential environmental and health risks of the product. Interference with animal reproduction can be mediated by toxicity to the gametes or earlier germ cell stages, or by toxicity to the embyro or larvae. These life history stages tend to be more sensitive than adult organisms, but no less critical to the survival of the species. Reproductive risk potential is usually expressed in terms of an EC50 or ED50 (effective

concentration or dose which inhibits reproduction by 50%). A low value indicates a high potential risk.

Teratogenic effects also represent a potential risk to health and environment. Teratogens interfere specifically with embryonic development. While they may permit reproduction, the offspring are deformed. In wild animal species, this is tantamount to reproductive inhibition, since few deformed offspring are able to survive to maturity. Teratogenicity is of particular concern from a human health standpoint since human society supports deformed individuals, and places considerable value on quality of life. Teratogenic potential may be expressed in terms of an EC50 or ED50 concentration or dose, or in terms of a teratogenic index (TI = LC50/EC50). A low EC50 or ED50 or high TI indicates a high teratogenic risk potential.

Mutagenic effects are of concern primarily from a human health standpoint, and particularly as they relate to carcinogenicity. With improvements in medical science and increased longevity, cancer, as a disease of later life, has become a leading cause of death. Many theories of carcinogenesis involve genetic damage, which can be measured by short-term tests for mutagenicity. A large battery of mutagenicity tests are available, using various test organisms or mammalian cell cultures (e.g., Ames et al., 1975; Searle, 1975; Preston et al., 1981). Concordance among these tests has recently been reviewed by the International Commission on Protection from Environmental Mutagens and Carcinogens (ICPEMC, 1983).

While most mutagens are also carcinogens, many carcinogens are non-mutagenic. Thus, mutagenicity and carcinogenicity do not necessarily represent the same risk factor. Specific tests for carcinogenicity have been developed. Long-term in vivo tests involve administration of the product to a test animal, usually a mammal, and subsequent examination of the animal for tumors developing later in life. Short-term in vitro tests involve administration of the product to mamalian cell cultures and subsequent detection of transformed (cancerous) cells.

Mutagenicity and carcinogenicity are both considered stochastic effects in that there is a finite probability of effect at any dose level or product concentration. There is presumed to be no concentration or dose level below which the effect cannot be induced and, if induced, it can be equally severe at any dose level. The probability of effect,

rather than the effect itself, is dose related. This aspect of the risk may be a significant factor in risk perception by the general public.

Allergenicity is primarily a human health consideration. Allergic reactions are caused by hypersensitization of the immune system. They are always uncomfortable and can be fatal. The risk of allergic reaction to a product can be assessed from hypersensitivity tests with mammalian test animals.

3.4.3 Environmental Fate

A chemical product released to the environment may persist indefinitely, or degrade as a result of chemical or biological oxidation. Environmental persistance is generally considered to increase the potential risk associated with environmental release. Persistant products tend to accumulate in the environment and, if at all toxic, may eventually reach toxic levels in the environment.

Products which degrade in the environment may leave chemical residues with quite different properties than the original product. These, in turn, should be assessed for persistance and toxicity in order to assess fully the potential environmental risk associated with the release of a given product.

Persistence is quantified in terms of the half-life of the product and its residues (the time required for 50% of the substance to be degraded). Environmental half-lives depend on environmental conditions, such as temperature, moisture and oxygen level, and should be determined experimentally for different environmental compartments (e.g., water, air, sediment) to which the product may have access. Biological half-lives may also be determined for substances which are taken up by plants and animals. These may differ from one species to another. Potential risk increases with half-life and toxicity.

Uptake by plants and animals in the environment poses the risk of bioaccumulation. This is the process by which a product or residues may accumulate in biota to levels exceeding those in the environment. The effect is quantified in terms of a bioaccumulation factor, which can by calculated at steady-state as the concentration in biota divided by the concentration in the environment. Bioaccumulation factors are specific to a particular environmental compartment (e.g., water, soil, sediment) and a particular species, and

may range from one or less, to 10^4 or 10^5. Bioaccumulation tends to be a function of chemical properties and may be roughly predicted for some chemical classes and biological taxa from chemical parameters such as the octanol-water partition coefficient (K_{ow}). Products or residues with a large bioaccumulation factor or large partition coefficient are usually considered to represent a correspondingly large potential risk to the environment and human health.

3.4.4 Genetics

The stability of a released organism's genome is an important consideration in assessing the risk associated with either intentional or accidental release of genetically modified organisms. An unstable genome is inherently less predictable than a stable genome and more likely to give rise to unexpected traits in the organism. Unexpected traits may arise through mutation, genetic transfer or recombination, and/or natural selection.

Mutations arise spontaneously in nature at a low rate on the order of 10^{-5} to 10^{-6} mutations per genetic locus per generation, although it is recognized that certain types of mutations are more likely than others, and certain loci are more susceptible to mutation than others. It has been argued that unexpected mutations should be of minor concern on the grounds that any mutations with sufficient survival value to persist in the genome will have arisen at some time in the past and, therefore, probably exist already in nature (Brill, 1985). However, this position is disputed by Alexander (1985) and Colwell et al. (1985). These authors argue on theoretical grounds that new genetic traits, particularly those based on multiple loci, are more likely to arise under protected laboratory conditions and that, once they become established in the genome, they can persist in nature and may alter the expression of subsequent spontaneous mutations. Such interactions within the genome are well documented, although they are somewhat in conflict with the traditional reductionist approach to cell biology (Newman, 1985).

Empirical evidence for significant changes in pathogenicity arising from small genetic changes is compiled by Alexander (1982). Perhaps the best example is the so-called swine influenza virus which appears to have arisen in recent times from a small change in an existing virus and has been responsible for millions of human deaths. It should be noted, however, that most of the examples given by Alexander involve changes in pathogenicity of organisms which were previously pathogenic, and strong selection pressure for

persistence of the genetic change (e.g., drug resistance). This emphasizes the point that the evolutionary relationships of an organism and current selection pressures in the environment are relevant to the probability of a hazardous mutation arising and persisting.

In view of the controversy among experts concerning the importance of mutational frequencies in risk assessment, it would be prudent to quantify these frequencies prior to release of a recombinant organism, particularly when evolutionary relationships to pathogenic or highly toxic organisms can be demonstrated. It would obviously be difficult to do this for every genetic locus in the organism, but emphasis may be placed on foreign genes and on genes known to affect other risk factors such as pathogenicity, toxicity or dispersal.

Genetic transfer is ubiquitous among living organisms, but particularly well developed among micro-organisms. In higher organisms, it is usually associated with sexual reproduction, and largely confined within species or groups of very closely related species. In micro-organisms, asexual mechanisms are more important. In bacteria, genetic exchange between organisms is completely separate from the reproductive process and not confined within species.

Genetic transfer and recombination within species is an important mechanism by which new characteristics may arise. It is difficult to predict which new characteristics are most likely, although genetic mapping based on meiotic crossover frequencies can be instructive in higher organisms. Genes which are closely linked on the same chromosome are least likely to separate as a result of recombination. Changes in relative gene position can alter expression of genetic traits. Also, changes in combinations of genes within the organism, regardless of position, can alter gene expression as a result of epistatic interactions.

Mechanisms of gene transfer between species are probably of greater concern. The presumed safety of a novel genotype in the environment is often contingent on the ecology of the host organism. For example, Bacillus thuringiensis with its gene for B-toxin persists poorly in agricultural environments; however, the same gene in Pseudomonas species which thrive in such environments may be much more difficult to contain.

Mechanisms of gene transfer between microbe species include transformation, transduction, plasmid transfer and conjugation. Transformation is the uptake of DNA from the surrounding medium (Goodenough and Levine, 1974). This process is a standard method of inserting DNA into an organism in the laboratory.

Transduction is the viral transfer of DNA into a bacterium (Stent, 1963; Thorne, 1974) or a cell of a higher organism. Viruses which attack bacteria are called bacteriophages. Many bacteriophages do not immediately kill the host cell, but incorporate their DNA into the host chromosome as a dormant prophage. The prophage eventually detaches from the host chromosome in order to synthesize infectious particles, and may incorporate some of the host DNA in the process. The new virus particles are then capable of transferring the host DNA to a new host of the same or a different species. It has been estimated that up to 30% of the DNA in some strains of E. coli may consist of prophages (Szybalski and Szybalski, 1974, cited by Reanney, 1976).

Plasmids are small circular pieces of DNA, usually separate from the main bacterial chromosome, which may have been derived from viruses that lost their infectious properties (Falkow, 1975, cited by Harwood, 1980). Like viruses, plasmids can sometimes exchange genetic material with the main chromosome, by attaching to and detaching from the chromosome. They differ in size, genetic composition, replication properties and host range, but are ubiquitous among bacteria. They are easily transferred from one bacterium to another, either by their own means or in connection with other genetic transfer processes. Conjugative plasmids contain genetic code for the formation of bridges, or pili, between bacteria, and can transmit copies of themselves across these bridges. Non-conjugative plasmids are transferred at much lower frequencies (John et al., 1981).

Each conjugative plasmid tends to have a limited range of host species to which it can transfer unaided. However, transposons often assist plasmids to cross species barriers that would otherwise restrict their movement (Nugent and Hedges, 1979). Transposons are genetic elements which promote the transfer of genetic material within a microbial genome, transfering plasmid (or chromosomal) genes from one plasmid to another. Thus, the collective host range becomes potentially available to all genetic elements.

Chromosomal conjugation is a mechanism for transfer of an entire donor bacterial genome into another bacterium, although only a portion of the transferred genetic material may be incorporated into the host bacterial genome (Goodenough and Levine, 1974).

Host ranges for genetic transfer of viruses, plasmids and chromosomes are determined by restriction/modification (res/mod) systems included in the genetic material (Roberts, 1976). The modification component codes for a protective labelling of a specific DNA sequence, such as by methylation. The restriction component codes for a restriction enzyme that cuts any unlabelled (unprotected) DNA into non-functional pieces. Donor and host res/mod systems are in conflict, as are the res/mod systems of different plasmids. Thus, certain plasmids are incompatible in the same host (Datta, 1979), and some hosts are protected against certain plasmids, depending on the relative efficiencies of the protective res/mod systems.

Rates of plasmid mediated genetic transfer are highly variable due to the complexity of the interactions that may be involved, but tend to be about 10^5 times greater than the rates of chromosomal transfer (Harwood, 1980). This appears to be true of non-conjugative, as well as conjugative plasmids, although conjugative plasmids have greater transfer rates. Dougan et al. (1978) show that specific genes on non-conjugative plasmids enable them to be co-mobilized to some extent, along with conjugative plasmids. These genes should be inactivated, if possible, as a precaution in genetic engineering with plasmid vectors.

Risks associated with accidental or intentional release of genetically engineered organisms depend upon the rates of genetic transfer of specific genetic elements, both into the released organism from wild species and into wild species from the released organism. The first type of transfer may be easier to assess since genetic monitoring could be limited to a single species (the organism to be released). However, the second type of transfer may be of greater concern since the organism to be released contains the novel and potentially hazardous genotype. Rate constants[1] for plasmid transfer

1 The rate constant multiplied by the product of donor and recipient concentrations equals the rate of transconjugant formation (cells/mL-hr).

ranging from 10^{-13} to 10^{-9} mL/cell-hr were measured by Levin et al. (1979) in exponentially growing E. coli populations.

Relative frequencies of transconjugants in equilibrium populations and communities are also used to measure the likelihood of genetic transfer. Levin et al. (1983) report a plasmid transconjugant frequency of about 10^{-4} transconjugants/recipient cell for a particular conjugative plasmid of E. coli in the human intestinal flora. Gainey and Davis (1978) report a similar value for frequency of transfer between E. coli and a totally unrelated anaerobic bacterium Bacteriodes. However, Sanderson (1976) reports that the transfer of chromosomal genes by plasmids is much more dependent on evolutionary relationship, citing a frequency of about 10^{-8} recombinants/donor cell in mixed populations of E. coli and S. typhimurium.

Genetic transfer rates and frequencies could be estimated in the laboratory under simulated environmental conditions. Engineered and wild micro-organisms would be combined in an artificial microcosm, and sampled periodically to determine densities and transconjugant frequencies. However, this requires a reliable method for identification of the inserted DNA. Labelled DNA probes, synthesized so as to hybridize with a particular complementary DNA sequence (Hill et al., 1984; Tatten et al., 1983; Murasugi and Wallis, 1984) could serve this purpose, and may also prove useful for monitoring genetic transfer in the field.

Characterization of an inserted DNA sequence should include estimates of gene transfer rates and potential recipient organisms, particularly when genes for pathogenicity or toxicity are involved, and particularly when plasmids are used as vectors during DNA insertion. Any known plasmids of the donor and recipient organisms should be catalogued, and their mobility determined, in view of their potential importance as genetic vectors. Known phages and their life cycles should be documented for the same reason. Organisms proposed for release without documentation of genetic transfer potential should be considered as high risk cases.

3.4.5 Ecology

The potential impact of any organism released into a new environment, whether genetically engineered or not, depends to a great extent on the ecology of the organism. Broad ecological considerations include the organism's ability to disperse from the

release site and colonize other areas, its interactions with other organisms and its role in the biogeochemical cycling of elements within the ecosystem. Relatively few organisms have been completely characterized from this ecological perspective, and inferences based on knowledge of closely related organisms are often made. In the case of a recombinant organism, an ecological understanding of both donor and recipient organisms is critical.

Dispersal of an organism from its point of application depends on the likelihood of release from the environmental compartment to which the organism is applied and transport to other compartments, the ability to establish and survive in environmental compartments to which transport is possible, and the potential for population growth in those compartments. Release involves the dislodging of the organism from its point of application, and is determined by physical location, mode of application, ambient rainfall, windspeed, and human or animal activity following application. Mechanical disturbance and aerosal release associated with rainfall was measured by Graham et al. (1977) at approximately 0.003% of organisms (Erwinia cantovora) on stems of potato plants over a 30-minute period (0.14% per day). Strauss et al. (1985) estimated release due to winds at about 0.1% of available organisms per day. Lighthart (1984) estimated that as much as 40% of aerial bacterial loading in the spring may come from mechanical disturbance by plowing. This is a potentially very important factor in agricultural applications. Insects, birds, earthworms or burrowing mammals may also contact inoculated plants and dislodge organisms (Gillett et al., 1984).

Transport of organisms once dislodged may be via air, surface water, groundwater or biological vectors such as passing animals, and may be either active or passive. Some microbes are able to actively swim in water, or catch the wind. Active movements are generally in response to concentration gradients of limiting nutrients. Thus, knowledge of concentration gradients may help to predict probable directions of active transport. Passive movements follow prevailing water flow or wind directions. Barnthouse and Palumbo (1985) have reviewed transport mechanisms and models for prediction of microbial transport.

Attachment to dust particles may determine the speed and range of passive aerial transport, smaller particles and unattached microbes having a greater range. Reports of particle size range from 1 to 5 um for unattached bacteria (Bovallius, 1980) to greater

than 8 to 9 um for attached bacteria and fungi (Lighthart et al., 1979; Jones and Cookson, 1983). Small particles are more likely to achieve heights which permit long-distance transport above the inversion layer. Bovallius et al. (1978) report transport of bacterial spores from the Black Sea to Sweden. Transport models which incorporate inversion layer effects, as well as microbial survival, are needed in order to reliably predict long-range aerial transport and transported population densities (Bovallius et al., 1980). Best available models are valid only to a distance of 1 to 10 km from source.

Surface water transport of organisms can be very rapid because of the high flow rates in some areas. Groundwater transport is much slower and may be influenced by organism motility. Some microbes can swim at 30 um/sec (Stanier et al., 1976). Adsorption onto soil particles can impede movements with groundwater (Evans and Owens, 1973), although this effect depends on soil saturation (McCoy and Hagedorn, 1979). Detailed groundwater studies are appropriate for released organisms which can survive in groundwater environments.

Survival and growth of micro-organisms will be specific to each micro-habitat. All habitats which the organism might contact should be considered. Organisms expected to survive and grow at the release point have essentially an unlimited time in which to be dispersed. Those with limited survival times will be much more easily contained. Survival can be estimated in the laboratory using microcosm test systems which contain other organisms and simulate the natural habitat (Pritchard and Bourquin, 1984). The same test systems can be used to estimate genetic transfer rates (see Section 3.4.4). Survival in various transport media (air, water) should also be determined. The standard measure of survival in air is the decimal reduction time (DRT) required for a 90% reduction in viability of airborne bacteria. A similar measure might be used in aqueous or soil microcosms. Aerial DRT's range from seconds or minutes for some pathogens to hours for some other bacteria (Dimmick and Akers, 1969; Anderson and Corf, 1967). Bacterial spores, if formed, may have much greater aerial survival.

The DRT should not be construed as an approximate time to elimination of an organism in the environment, since population decline, when it occurs, tends to be an exponential phenomenon. This pattern of decline is illustrated by Karns et al. (1984) for a Pseudomonas species developed for toxic waste cleanup. The 10% of organisms which survive longer than the DRT may persist for long periods. However, the population survival time is probably correlated with the decimal reduction time.

Other introduced populations may grow after release, and can supplant similar species if given a slight competitive advantage. Competitive advantage may depend, among other factors, on the size of the inoculum. Weller (1984) illustrates this phenomenon with a Pseudomonas species replacing other organisms on plant roots. Such replacement is the objective of some biotechnology projects (for example, release of the "ice-minus" bacterium). Alexander (1981) reports that some bacteria tend to maintain a predictable soil density, given time to achieve this stable population level, whereas other species may be much less predictable.

Survival and growth in natural (or simulated) ecosystems usually depends on interaction with other organisms. Interactions may be complex and multifactorial, with the interaction between any two species depending on the other species present, as well as on resource availability. Even in artifically simple two-species systems, the outcome of a contest between two competitors may depend on a host of environmental conditions which favour one species or the other, and on the initial densities of those species (i.e., size of inoculum). Interactions tend to be homeostatic (i.e., they stabilize the ecosystem, permiting it to compensate for perturbations such as introduced species). However, this does not imply that ecosystem changes in response to perturbation will be insignificant. Overall ecosystem function tends to be preserved, but this may be at the expense of individual species.

Interactions between pairs of species may be categorized in terms of competition, predation, parasitism, mutualism (both species benefit), commensalism (one species benefits) or amensalism (one species is adversely affected). However, the relationship between two species may change with the addition of a third (i.e., from predation to competition) or with changes in the physical environment or resource base. Thus, two-species models are very crude representations of nature. Even multi-species models have a very poor record in predicting effects of new species introductions (Simberloff, 1981).

Paradoxically, predation within the community seems to have the effect of maintaining prey species diversity. This is because predation tends to keep populations of competing prey species at low levels that do not strain food or habitat resources. Alexander (1981) notes that microbial prey species are seldom completely eliminated by predators, and attributes this to the concept of prey-switching; predators switch to feeding on alternate prey species whenever the density of one prey species declines to a critical level.

Empirically, minority species, if able to reproduce at all, tend to persist in the environment at very low levels, and populations may recover if conditions become more favourable. Liang et al. (1982) discuss this phenomenon with reference to microbial species in sewage systems. Alexander (1985) cites two good examples: Agrobacterium, a plant pathogen, and Rhizobium, a nitrogen-fixing bacterium important in genetic engineering. Both organisms persist quite well at low levels in certain natural soil micro-habitats, even though neither organism forms resistant structures.

Empirical evidence suggests that a small proportion of species introduced to new environments result in extinction of resident species (Simberloff, 1981), although Greenway (1967) reports resident extinctions in about 30% of cases studied. It is always difficult to document the introductions that failed, and these may be expected to be underestimated. Nevertheless, cases of introduced micro-organisms which have adversely affected man in some way are well documented. Alexander (1985) lists a number of examples such as Dutch Elm Disease (Ceratocystis), Chestnut Blight (Endothia), Corn Leaf Blight (Helminthosporium), and swine influenza, which appears to have arisen as a slight genetic change to an existing virus. Sharples (1982) reviews the literature on introduced species, and concludes that the probability of ecosystem disturbance by a novel genotype can only be estimated by experimentation with the novel organism in model ecosystems.

In view of the complexity of community dynamics, historical experience with novel species, and the incomplete ecological saturation of many communities (Collwell et al., 1985), arguments that engineered organisms pose negligible environmental risks on the grounds that organisms capable of prolonged survival probably already exist in nature (Brill, 1985) are not convincing. As pointed out by Collwell et al. (1985) and Alexander (1985), while probabilities of adverse environmental impact may be small, they are not zero, and warrant a serious effort at risk evaluation in cases where environmental release is contemplated.

Microbial effects on biogeochemical cycles are also of potential concern in environmental applications of genetically engineered or other introduced organisms. Every element cycles through the ecosystem from a geochemical reservoir, to biota, from one living organism to another, and finally back to the reservoir when organisms die and decompose. Bacteria and fungi play a very important part in such cycles since they provide the main link between biological and geochemical compartments.

The phosphorus cycle is a good example of how man's activities can have a disruptive effect. Phosphorus is an essential nutrient for most life forms, and is typically in short supply. It is considered to be a limiting nutrient in most ecosystems. The main reservoir is in rocks and natural ground deposits. Man takes phosphorus from these reserves for application as fertilizer in agricultural fields. The fertilizer is required to replace phosphorus which is removed with the crop each year. Surface water runoff carries much of this added phosphorus into lakes and rivers (where algal blooms reflect the sudden release from phosphorus limitation) and eventually into the ocean. Sedimentation in the ocean results in deep-sediment burial of most phosphorus mobilized by man, since phytoplankton activity is inadequate to keep this amount of phosphorus circulating. Currently, more phosphorus is lost to marine deep sediments than is returned to terrestrial and freshwater ecosystems (Hutchinson, 1957).

The nitrogen cycle (Figure 3.5) may be particularly sensitive to alteration by release of genetically engineered organisms, since the nitrogen fixing bacteria are intimately involved in that cycle, and are the subject of intensive recombinant DNA research. Nitrogen fixing bacteria, such as Rhizobium, Azobacter and Clostridium, reduce atmospheric nitrogen to ammonia. Nitrifying bacteria, such as Nitrosomonas, oxidize ammonia to form nitrite. Other nitrifying bacteria, such as Nitrobacter, oxidize nitrite to nitrate. Plants utilize either ammonia or nitrate for protein synthesis. Denitrifying bacteria, such as Pseudomonas, and fungi, convert nitrite, nitrate and ammonia from decomposition or excretion of higher organisms, back to gaseous nitrogen. The atmospheric concentration of nitrogen is a result of the balance between these processes. Attempts to increase nitrogen fixation on a large scale, using genetically improved micro-organisms, might potentially alter the kinetics of the cycle; however, denitrification would likely compensate over the long-term.

The balance between such opposing biogeochemical processes should be carefully considered as part of the risk assessment process for release of a novel organism. This is particularly important in environmental applications which are intended to enhance metabolic processing such as enhanced nitrogen fixation or mineral leaching. The micro-organisms involved in mineral leaching (e.g., Thiobacillus ferrooxidans) are sulphur oxidizers and should be considered in relation to the sulphur cycle (Figure 3.6). The sulphur cycle contains both atmospheric and marine sedimentary reservoirs. Eriksson (1963) provides a global mass balance, and shows that the oceanic compartment is

FIGURE 3.5: THE NITROGEN CYCLE

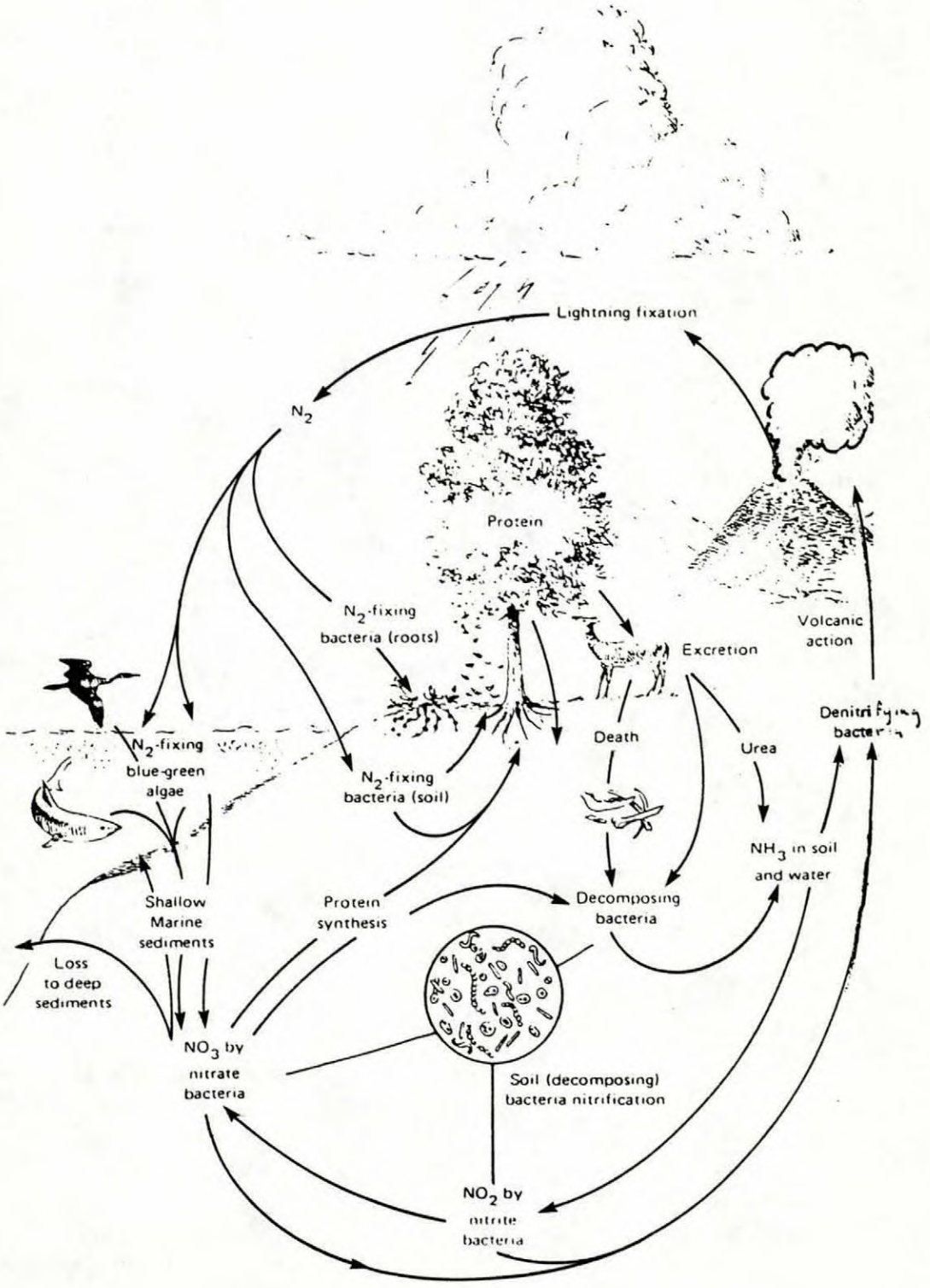
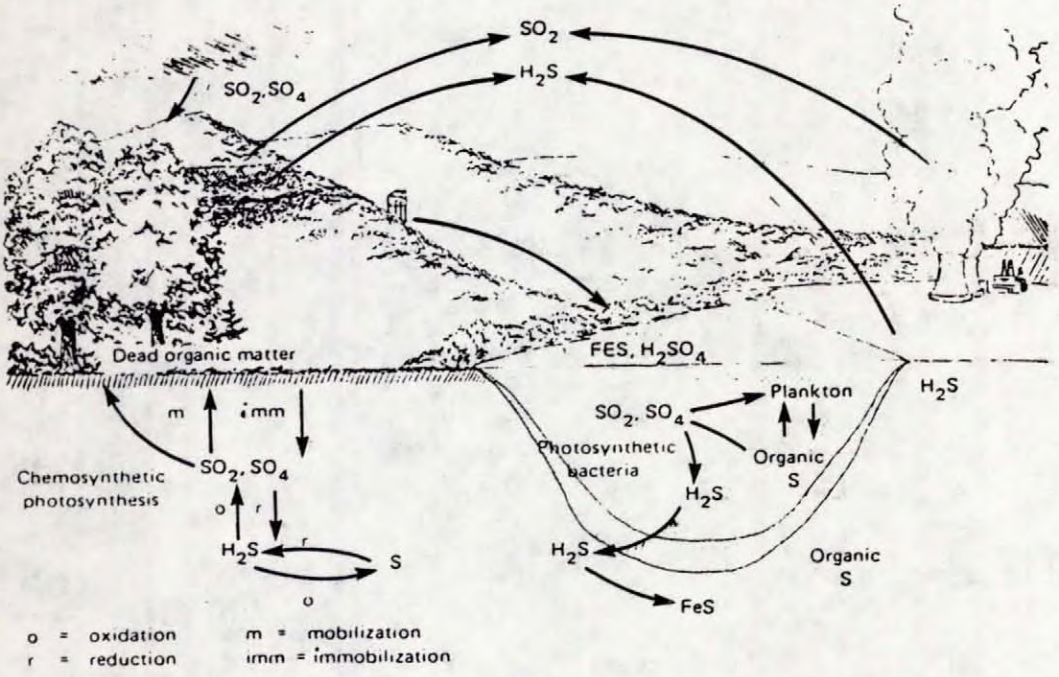


FIGURE 3.6: THE SULPHUR CYCLE



currently being enriched with sulphur at a rate of about 50 million tons per year. Oceanic enrichment is probably due mainly to industrial emissions and fertilizer applications.

3.5 Risk Assessment

Risk assessment includes identification of potential hazards and hazardous agents, estimation of human and other organisms exposure to those agents, estimation of dose-response relationships, and estimation of the incidence of adverse effect based on those relationships. Fisher (1985) reviews the history of risk assessment in the context of recombinant DNA research, and concludes that, while perception of risk associated with contained facilities has decreased over the last decade, this has been based more on a ten-year experience without detectable hazard than on conclusive experimental evidence concerning organism release, survival or establishment. Recent proposals for intentional release of genetically altered organisms in the U.S. have raised renewed concerns.

From the preceding discussion of potential risk factors, a list of basic information requirements related to risk assessment may be compiled. The list in Table 3.3 points to broad information categories, each of which could be further subdivided. Examples of pertinent information input under each category have already been discussed. Ultimately, the specific questions to be asked under each category will be decided on a case-by-case basis as each proposal for release is evaluated. This may well involve several rounds of information input as increasingly specific questions are formulated based on previously supplied information. Sutter (1985) lists the components of a formalized risk assessment process, including:

- criteria for determining information requirements;
- a set of test systems to obtain the information;
- a set of rules for interpreting test results; and
- models for illucidating the implications of test results.

Information requirements fall logically into three tiers. If the product is to be contained in a laboratory and not transported, demonstration of those containment measures is a first priority. Present MRC guidelines seem to be suitable criteria against which to judge the adequacy of proposed containment measures. Proposals meeting these criteria may need no futher evaluation.

TABLE 3.3: INFORMATION REQUIRED FOR RISK ASSESSMENT PURPOSES

Product	Laboratory	Field[2]
Chemicals	Toxicity[3]	Toxicity[5,6] Acute lethality Chronic lethality Reproductive effects Allergenicity Teratogenicity Mutagenicity Carcinogenicity Fate[5,6] Residues Persistance Bioaccumulation
Biologicals[1]	Strain History Pathogenicity Containment[4]	Pathogenicity[6] Infectivity Virulence Host Range Vectors Genetics[6] Inserted sequence Known loci Mutation rates Known plasmids Transfer frequencies Plasmid host ranges Dispersal[6] Release mechanisms, rates Transport media, rates Survival time Growth, establishment Interactions[6] Predators, prey Competitors Parasites, hosts Biogeochemical Role[6] Metabolic processing

1 Requirements in addition to those for chemicals.
2 Requirements in addition to those for the laboratory.
3 Provincial responsibility, research and development may be exempt.
4 As per MRC guidelines in the laboratory.
5 Environmental Contaminants Act applicable but lacks pre-market notification requirements.
6 Pest Control Products Act and Food and Drug Act have pre-market notification requirements.

Toxicity and environmental fate information is appropriate for any product which is intended for environmental release, medical use or transport, whether the product is considered living or non-living. Several problems may arise in requiring such information. The most formidable problem is the cost involved in obtaining the full range of data. Costs can be minimized by starting with the least expensive tests, such as acute lethality, and stopping when the product fails a test. However, even testing at this first level may become a formidable task by virtue of the sheer number of products to be tested (Maugh, 1978). Various prioritizing schemes have been suggested for identifying chemicals most in need of further testing, although no such scheme has been formally adopted. It is doubtful that biologicals are yet sufficiently well understood to permit development of such a scheme.

Apart from the cost involved, the problem of when to require testing must be addressed. Every product goes through a developmental progression from basic research, through bench-scale production, to pilot-plant, and finally full-scale manufacturing. Licensing is seldom required until the final stage, if at all, yet the pre-manufacturing risks may be significant.

Pathogenicity, genetic and ecological information are appropriate for any product intended for environmental release, medical use or transport, and containing living organisms. Although strictly speaking, viruses are not living organisms, because of their potential to infect other cells and to multiply, they should also be considered in this context. If a product can be demonstrated not to contain living organisms, exemptions from this phase of testing and documentation may be considered. However, conclusive demonstration that a product does not contain living organisms can be difficult since detection to the level of a single micro-organism is not possible (Harrison and Hattis, 1985). Moreover, even naked DNA not contained within an organism can be assimilated by some micro-organisms in the laboratory, and presumably also in the environment. Thus, a more relevant test of the product at this stage may be a test for DNA. This also avoids semantic debate about the definition of life.

A conservative approach, recently proposed by the U.S. EPA, is to designate any product of a biotechnology process as subject to full information requirements, at the discretion of the evaluation team. The efficacy of this approach hinges on a very detailed and unambiguous definition of biotechnology.

Products considered to contain DNA or living organisms and intended for environmental release should probably be evaluated in several stages, just as medical products are required to go through animal testing followed by clinical trials. In environmental risk assessment, a first round of testing can be completed in the laboratory using microcosm tests (Pritchard and Bourquin, 1984) to simulate the environment and provide preliminary estimates of genetic transfer, population survival and ecological interactions. Standard test protocols and pass or fail criteria would have to be defined. The results of such tests, along with complete genetic, evolutionary and ecological characterization of the product, would determine whether field trials are performed.

Mechanisms of dealing with proprietary information at this stage will be essential. It is important that comprehensive genetic information be considered in the evaluation, particularly for organisms containing foreign DNA, and this information will be considered proprietary. It cannot be made available to the public or competing companies as this would discourage full disclosure.

Products approved for field testing should be closely monitored in the environment. Monitoring methods used in microcosm tests to detect the introduced organisms and measure genetic transfer to other organisms may also be applicable in the field. These include fluorescent monoclonal antibodies or labelled DNA probes (Totten et al., 1983; Hill et al., 1984). Test boundaries should be clearly defined, and containment measures designed to keep the introduced organism and genetic material within those boundaries should be described. Control measures which might be taken to curtail the experiment if necessary, and conditions under which those would be implemented should also be clearly defined in field trial proposals. Control measures might include conditional lethal mutations engineered into the organism. Temperature sensitive mutations, for example, could be activated by warming or cooling the test area, although some spontaneous reversion of such mutations to the wild type is expected (Page et al., 1985). Soil sterilants might also be used. However, Strauss et al. (1985) emphasizes that complete control is extremely difficult to achieve in an environment that favours growth and dispersal.

Containment strategies which isolate the field test area from dispersal forces may include plastic liners of the type used in landfills. Physical barriers are most likely to be useful in small areas. Biological barriers might include elimination of genes for

sporulation, or for production of some rare essential nutrient that would then be supplied within the boundaries of the test area (Blattner et al., 1977). Harrison and Hattis (1985) review containment strategies for greenhouse trials in some detail.

Environmental monitoring for introduced organisms and their genes during field trials should include air, soil, surface water and groundwater. Field trial results compared to microcosm test results will likely provide insight into the reliability of microcosm testing, and may suggest improvements to the protocol as data accumulate. Field trial data may also help to refine mathematical models for prediction of environmental impacts based on microcosm and other available data prior to release.

4.0 CURRENT REGULATORY APPROACHES AND STRUCTURES

4.1 Regulatory Approaches

Regulatory approaches to biotechnology in other countries suggest various models on which a Canadian approach could be based. Specific details and recent developments in each country are given in the international overview (Section 2.1). Several generic models and their relative merits, based on international experience, are discussed as options in this section.

4.1.1 Voluntary Guidelines

The simplest and most widely used approach at the present time is the voluntary guidelines system administered by research funding agencies in many countries. The MRC guidelines are the current Canadian standard for laboratory research. No Canadian standard exists for intentional environmental introduction of biological products produced using new biotechnologies. The advantage of guidelines over legislative instruments is their flexibility. Guidelines are easily changed and updated in response to changing directions in biotechnology and improved understanding of the risks involved. The disadvantage of guidelines is that they are difficult to enforce. While government funding agencies have considerable influence in basic research, support from private sources becomes more prevalent in applied research and development, particularly at the scale-up and production stages where releases to the open environment are likely to be more common-place.

Mandatory guidelines are a misnomer. While they may be intended to apply broadly to both privately funded industry and government funded research, a simple statement to this effect within the guidelines is not sufficient to ensure compliance. If legislation is used to enforce compliance, the legislation will specifically incorporate and subsume the guidelines. 'Blank check' legislation, giving the force of law to guidelines that may be revised in future by non-regulatory bodies, is not a practical alternative.

4.1.2 Certification Systems

Certification systems can have the flexibility of guidelines, while providing a strong incentive for compliance. Non-regulatory bodies, such as professional societies, can

issue certificates of approval based on adherence to guidelines. While they may not have legal authority to enforce codes of practice, a high-profile creditation system can carry its own incentives, based on professional reputation and its role in business. The Swiss Academy of Medical Sciences Commission for Experimental Genetics operates on this principle with apparent success.

4.1.3 Specific Legislation

Guidelines may be entrenched in law as new legislation. The Federal Republic of Germany has recently undertaken this change in its approach to biotechnology. However, the practicality of this approach depends upon sufficient experience to assess the legislative requirements. The last decade of experience in biotechnology research laboratories seems to suggest that research laboratory guidelines are adequate, and could be legislated if necessary, although some time would be needed to put the legislation in place. On the other hand, there is so little experience with environmental introduction of genetically engineered organisms, and so much uncertainty as to the appropriate degree of regulation necessary, that specific legislation in this area may be premature.

4.1.4 Licensing Systems

Licensing systems have the flexibility of guidelines while maintaining the force of law. The licence is required by law, and penalties may be imposed for unlicensed operation. Adherence to guidelines may be considered in granting or denying the licence, although the guidelines themselves are not incorporated into the legislation. The licensing agency may amend its guidelines or licence conditions as dictated by experience with the new technology.

In many countries, the key federal legislation which has been identified as applicable to biotechnology assigns a licensing authority. In the U.S., the FDA licenses human foods and drugs, the USDA licenses seeds, animal feeds and veterinary drugs, and the EPA has assumed licensing authority for most other environmental introductions. In Canada, Health and Welfare and Agriculture Canada assume licensing authority similar to that of the U.S. FDA and USDA. However, Environment Canada does not have a licensing authority by which to regulate environmental release, nor are biologicals included in its mandate. An amendment to the Environmental Contaminants Act would be required to

make effective use of licensing for environmental release of some classes of products produced using the new biotechnologies. In this regard, Environment Canada has proposed a new Environmental Protection Act, and consultations are ongoing as to its coverage of biotechnology processes and products.

4.2 Regulatory Structures

The division of regulatory authority that applies to certain products or processes of the new biotechnologies may lead to inconsistent treatment of applications which happen to fall under different jurisdictions. It may also lead to jurisdictional disputes between agencies. This could be particularly true in Canada where the provinces play a major role in environmental protection. Several optional regulatory and coordination support structures are described in this section, with specific reference to this potential problem.

4.2.1 No Formal Coordinating Structure

This option is closest to the current Canadian situation where a number of agencies at the federal level (Environment Canada, Agriculture Canada and Department of National Health and Welfare), and corresponding agencies in the provinces, share jurisdiction. While these agencies can and do communicate, there is no formal structure to ensure consistent coordinated action and information gathering, or to resolve jurisdictional questions.

4.2.2 Coordinating Office without Regulatory Responsibility

In the U.S., the Biotechnology Science Coordinating Committee (BSCC) will act as a coordinating body, without any role in project approval. Proposals are received and approved by the regulatory agencies, and summary information is passed on to the coordinating committee. This committee provides an interagency information monitoring service, compiling data on environmental introductions, adherence to guidelines and regulatory actions, and advising the regulatory agencies of any regulatory inconsistencies or potential environmental problems that arise. Representatives of the key regulating agencies, universities and industries could be included on the committee, with appropriate constraints on confidentiality. Such a committee could provide a certification service, in addition to its information gathering and dissemination, but would not have a licensing authority.

4.2.3 Coordinating Agency with Limited Jurisdiction

An alternative solution to the problems of regulatory consistency and jurisdiction lies in the formation of a coordinating agency to monitor developments in the field without usurping or duplicating the functions of other agencies. In Australia, the RDMC assumes this role, in addition to its role in review and approval of laboratory research proposals. Proposals for environmental release are reviewed and passed on to appropriate regulatory agencies for approval. The 'single-window' coordinating agency simplifies the approval process from the industry's perspective. Its membership can include representation of the key regulatory agencies, the universities and the industrial sector, all subject to legislation protecting the confidentiality of information received. The mandate of this agency could include licensing or certification in areas not covered by other regulatory bodies, as well as an information compilation and dissemination function.

4.2.4 New Agency with Full Jurisdiction

The most comprehensive approach to the jurisdictional problem would be to create a separate regulatory agency for biotechnology, with authority over biotechnology products and/or processes, however these are defined. Such an agency would usurp the authority now vested in other agencies whenever biotechnology was involved. It is doubtful, however, that a new agency could become functional in less than five to ten years. A decision-making mechanism to deal with proposed environmental introduction would still be required in the interim. This option would offer the potential of ensuring that no regulatory gaps exist, but it would do so at the expense of possibly replacing jurisdictional responsibility in several agencies which are very competently and efficiently regulating well-defined areas. There would also still exist broad areas of jurisdictional and trained manpower overlap that would be impossible to eliminate.

4.3 A Possible Regulatory Coordination Concept

Based on the review of the present regulatory and jurisdictional structure which exists in Canada, and in view of the regulatory response that has been taken in other countries with emerging biotechnology industries, it is the authors' opinion that the appropriate Canadian response at this point in time would be the formation of a Biotechnology Coordination Office that does not have regulatory responsibility, similar to the BSCC in

the U.S.A. Because the office does not have any regulatory responsibility, it is imperative that it operate with the full cooperation of all appropriate federal and provincial regulatory agencies. If this cooperation does not exist, the purposes for which it is proposed will not be achievable.

In Canada, the parlimentary and judicial system is better suited toward a consultative rather than adversarial system of review. With a consultative system, polarization may be avoided and some degree of common philosophy may emerge upon which regulatory controls can be based. The development of a common philosophy of approach, incorporating both federal and provincial as well as an overall public understanding and acceptance, will depend upon a broader knowledge of both the benefits and risks of biotechnology than presently exist. This knowledge will, in turn, only be gained through experience as several products of the new biotechnologies move from process development through commercial application with associated benefits, both in Canada and world-wide. As biotechnology and its products become 'familiar' to the Canadian regulatory infrastructure and the Canadian public at large, areas that require legislation will become apparent. This process will be augmented by observation of developments in other countries. Because of the rapidly evolving nature of the biotechnology industry in Canada and internationally, it is expected that a period of at least five years will be necessary before the data necessary for the justification and formulation of specific legislation will be available.

In the interim, the Canadian biotechnology industry needs some clear guidance as to what regulatory requirements certain types of products are expected to comply with, and the Canadian public needs to be assured that any research, development and environmental introduction of the products of the new biotechnologies are carried out in a safe and prudent manner. In the developmental phase of the biotechnology industry in Canada during which the roles of particular regulatory agencies and government departments are being defined, it is imperative that private industry have a 'single window' government agency or office which can serve a coordination role between industry and the federal and provincial governments. For convenience, this 'single window' biotechnology coordination office is referred to in this section as the BioCor office.

This concept of a single window coordination office with no regulatory powers but a strong mandate for information gathering and dissemination is perhaps closest in an international context to the approach adopted in the United States. The United States

has proposed the creation of a similar office to Congress. The U.S. equivalent office was initially proposed to have some regulatory power, but the jurisdictional overlap that became apparent upon close scrutiny resulted in the office having been stripped of any regulatory authority.

The creation of a Biotechnology Coordination Office in Canada will involve the adoption of several policy statements and implementation options previously outlined in Section 2.0. The policies which this office implicitly adopts and the policy options that it would be required to implement may be summarized as follows.

- The office would need to have by design a high degree of flexibility in dealing with jurisdictional and technical biotechnology issues on a case by case basis and this necessitates that a government policy statement allowing its creation be general in its wording.
- Because, by their very nature, the products of the new technologies cut across a wide range of regulatory jurisdictions, and because there is a wide range of potential public health and environmental concern that may be associated with the broad spectrum of products that can be introduced into the environment, the office must be able to handle applications on a case-by-case basis and have completely open channels of communication with the appropriate regulatory agencies.
- Since the primary role of the federal government is assumed to be in the area of ensuring the protection of public health and the environment the biotechnology coordination office needs to have the full cooperation of appropriate regulatory agencies to ensure that gaps in the present regulatory structure are fully recognized and taken into account until such time that it can be demonstrated that if these gaps are left they pose no significant risk to public health and environment or that they pose a significant risk requiring appropriate legislation to fill them.
- There is a need for the coordinated development of testing and evaluation protocols for the products of the new biotechnologies in Canada and this office should have as part of its mandate the ability to ensure, through its regulatory coordination function or independent research through the office, that these are developed on a national scale with regionally consistent risk evaluation and acceptance criteria for the research, production and field testing stages.

- There is a need to build up a central data base of both Canadian and international experience with products of the new biotechnologies in the research, production, field testing and open environmental introduction areas. In order that this coordination office has the cooperation of appropriate regulatory agencies, and in order to be aware of all aspects of the new biotechnologies being carried out in Canada, there needs to be a notification requirement, possibly through this office to the appropriate regulatory agency, at appropriate stages of research, development and testing, as well as feedback to the office on market use.
- If certain areas of the new biotechnologies are to be actively encouraged or prohibited, this coordination office should be aware of these policies and be in a position to communicate them clearly to industry upon receipt of notification.
- As part of a public consultative process in the area of biotechnology the coordination office should be in a position to ensure that the affected and interested public is fully aware of the benefits and risks of new applications which may affect them.

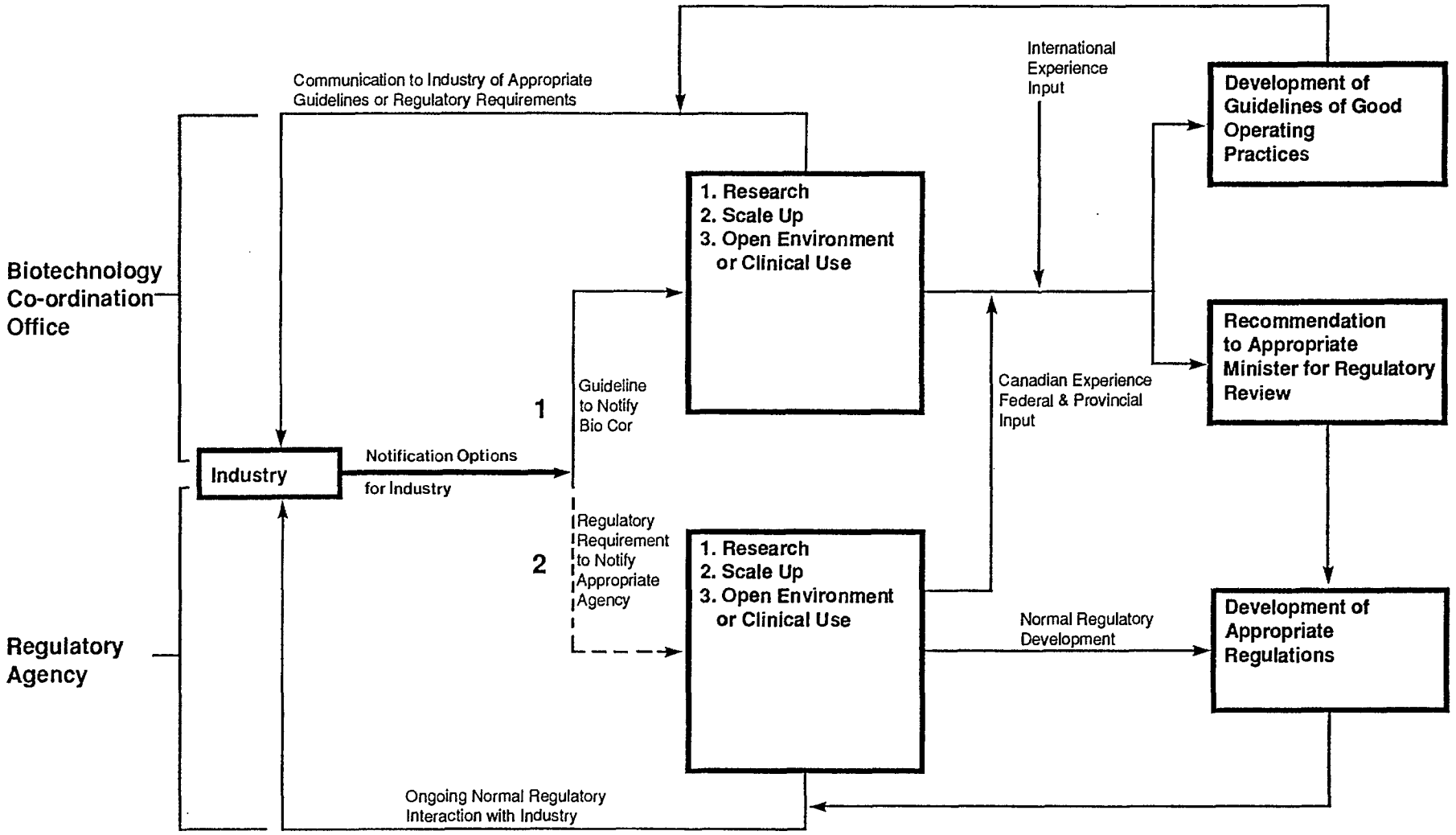
4.3.1 Biotechnology Coordination Concept

This office is proposed to have a core staff of professionals supported by administrative staff and contracted outside expertise as required. The professional staff may be seconded from agencies within government which currently have expertise and potential regulatory authority in the area, or staff may be hired from the private sector. A technical ratio of two individuals with regulatory backgrounds and a working knowledge of the government system at the federal or provincial level, to one member with some industrial experience, would be optimal. This office would not usurp the regulatory authority of other agencies, but would act as a notification point and coordination office for various phases of biotechnology research and development. The function of this office is shown schematically in Figure 4.1.

4.3.2 Biotechnology Coordination Office Mandate

The mandate of the Biotechnology Coordination Office (BioCor Office) may be outlined as follows:

FIGURE 4.1 A Biotechnology Co-ordination Office (Bio Cor) Interaction Concept



- To provide a coordination and notification function that serves as a link between various federal and provincial government regulatory agencies and research groups carrying out privately and government funded biotechnology research in Canada. This would include groups carrying out research and development employing rDNA or cell fusion technologies, or that are involved in the large scale production of naturally occurring plant or animal pathogens or other species which may pose a demonstrably significant risk to human health or the natural environment.

- Within this capacity this office shall:
  1. Require notification of projects in terms of a project proposal outlining the nature of the work to be carried out and the objectives to be achieved. Included in the notification would also be the name of the Biotechnology Safety Officer responsible for the project and a short description of the safety officer's qualifications and duties as they relate to staff training and knowledge of the risks that may be associated with the project.
  2. Advise the proponent on the training of their personnel with respect to safety procedures and guidelines to be followed to ensure the protection of human health and environment.
  3. Consult with the appropriate regulatory agencies, and advise the proponent of the regulatory agencies at a federal and provincial level that the proponent should work with at various phases of the project development. Assure that the proponent is aware of current regulatory controls, testing protocols that will be required, and the criteria on which test results would be evaluated. The office may also, based upon its experience with other similar Canadian or international projects and dialogue with the appropriate regulatory agency, advise the proponent of any potential roadblocks or time delays which may arise in the approval process based upon the degree of detail provided in the notification.
  4. Establish contacts and maintain liaison with monitoring bodies in other countries in order to exchange non-proprietary information and build an international data base upon which regulatory agencies can draw in providing approvals.
  5. Work closely with and coordinate the work of groups such as the Medical Research Council or other recognized agencies in creating and or updating

guidelines or appropriate standards for carrying out various types of research and testing.

6. Assume responsibility in conjunction with the appropriate regulatory agency for the creation of a series of ad hoc evaluation and review committees which will contain, for cross-agency continuity of decision making, members of the Biocor Office. The function of these review committees is to establish protocols and evaluation criteria and to evaluate data and specify reporting conditions necessary for the marketing and release of organisms covered under the office's coordination and notification mandate.
7. Report to the minister responsible on an annual basis on the status of the activities of the office and make recommendations as appropriate that may relate to a changing of the mandate of the office in order to achieve a better coordination and liaison role between industry and governmental regulatory bodies.

In practice, it is proposed that industry would approach the BioCor office at a series of product development stages from the initial intent to carry out research in certain advanced biotechnologies through to the field trial, marketing and product application stages. The office is not meant to displace existing regulatory controls where they are at present adequate, but rather to coordinate between industry and the appropriate regulatory agencies, and assist industry in defining the best approvals pathway. Where there are regulatory gaps, its function is to identify these gaps to the appropriate regulatory agency and, in conjunction with the appropriate regulatory agency, monitor the situation until enough hands-on-experience is gained that it can be determined if specific regulatory instruments are necessary to fill these gaps. After notification by industry, BioCor would approach the government departments which have a mandated interest in the particular activity that is proposed to be carried out. If an adequate regulatory control mandate already exists, BioCor would specify this to industry, set up the necessary series of contacts, and then allow the normal flow of regulatory process to occur between industry and the appropriate agency. From this point on, the office would be informed (notified) of developments from an information and coordination standpoint only. If an appropriate regulatory mandate cannot be found to apply, then BioCor would recommend the most appropriate vehicle for a licensing or certification function or guidelines to be followed until the need for specific regulatory control is demonstrated and appropriate legislation passed. If experience in Canada and elsewhere demonstrates

over time that the present or modified Canadian regulatory system is adequate and functioning well, then the BioCor office mandate would be reviewed and modified as appropriate. Part of the mandate of BioCor is to assess whether or not the regulatory mandate, staff experience and manpower are adequate in various areas and to make recommendations for changes where appropriate.

4.3.3 Specific Functions of a Biotechnology Coordination Office

The principal purpose of the BioCor office is to provide one window advice to the biotechnology industry in Canada as to what regulatory requirements exist at any particular stage of product development. This advice should be as specific as possible, dependent on the degree of disclosure industry is willing to make, in confidence to the office, at any particular stage of development. At this point, we would envision three distinct notification stages for industry to the BioCor office. These are briefly discussed in the following sections. At any time, however, any group may approach BioCor for clarification on what notification requirements and regulatory controls it may be subject to at any stage in product research, development, marketing or sales. The interaction of the BioCor Office with industry and appropriate regulatory agencies at various stages of project development is discussed below.

Stage 1 - Research and Development

This is the initial notification stage where a government, educational or private industrial laboratory would notify BioCor and inform them through a brief one-page project proposal format of their interest or intent in carrying out advanced biotechnology research in Canada. BioCor would then respond in one of two ways, depending on the outcome of subsequent communication with appropriate regulatory agencies.

If the nature of the research to be carried out falls clearly, from the initial research stage through to the product marketing stage, under the regulatory mandate of an existing regulatory agency or agencies, then BioCor would act as a coordinator ensuring that the company and the agencies concerned have communicated and that industry is aware of what is required under the regulatory mandate of the agencies. BioCor would issue a notice to the appropriate regulatory authority or authorities advising them that the proponent is aware of health and environmental safety precautions that need to be

followed, confirming that notification had occurred, and would then cease to have an active role in the project. A passive role would continue in that the regulatory agency responsible would keep BioCor as the central registry agency informed of developments in research and eventual scale-up and marketing. This information would then be cross-referenced in the BioCor file in order that BioCor may have access to the data coming out of the project, in order to assist it and other agencies in the evaluation of similar projects.

If the nature of the research is such that no regulatory agency has a clear mandate for regulatory responsibility, then BioCor will issue an advisory to the company or individual advising them that assignment to an appropriate regulatory authority is not appropriate at the present time, and that research can proceed, conditional on the company or individual carrying out the proposed research within the appropriate guidelines for advanced biotechnologies research. BioCor will then assume responsibility for monitoring developments in the area and making recommendations as to whether or not specific regulatory controls are required.

Stage 2 - Commercial Scale-Up

If the research and development appears to be leading to a product, the production of which may be scaled up to a commercial scale with the possibility of being marketed in Canada or abroad, then before proceeding from the research and development stage to a scale-up stage, the industry is responsible for notifying BioCor of its intent in this regard. At this point, BioCor would, as a first step, canvas the appropriate regulatory agencies to determine if any agency other than the agency or agencies involved in Stage 1 has a clear mandate to oversee the scale-up and production of the product. If full regulatory coverage is available through another agency, BioCor would advise both the company and regulatory agencies concerned and, through the appropriate agency, ensure that appropriate health and safety precautions are being followed. BioCor would then be kept informed by the appropriate regulatory agency on an annual basis of developments in the area.

If no clear regulatory mandate exists or only a partial mandate exists, BioCor would assemble a review committee of seven persons. Three would be BioCor staff, three would be outside experts and one would be a lay member of the community in which the

activity was to take place. The BioCor members would, based on international and previous experience, draw up a set of guidelines to be followed for the scale-up and production of the product. These guidelines would then be reviewed by the seven-person committee and either accepted as submitted or revised prior to acceptance. The guidelines would then go to industry for their review prior to becoming applicable to the activity. If, in the opinion of the BioCor committee, there is a 'clear and demonstratable danger' associated with the planned activity that is not covered by current regulation, then BioCor would make a recommendation that consideration be given to passing legislation or broadening the interpretation of an existing regulatory mandate enabling the activity to be regulated by a specific regulatory agency.

Stage 3 - Introduction to the Open Environment

At any stage during the scale-up process, when industry sees a possibility of marketing a product derived from the use of a biotechnology falling within the oversight area of the office, they can notify BioCor of their intent to distribute and market the product. This would constitute a pre-marketing notification. If a regulatory agency has a clear regulatory mandate for overseeing the marketing and distribution, BioCor would then treat this as a notification requirement only and, upon demonstration to BioCor through the mandate of the regulatory agency concerned, that appropriate health and environmental safety standards are being met, simply acknowledge receipt of notification. If no such mandate exists within a regulatory body, BioCor would respond with a request for data necessary for BioCor to discuss with appropriate regulatory agencies whether licensing is required and, if so, whose responsibility it would be to issue a licence and what the conditions of that licence would be. If the data presented suggest that a licence for marketing and distribution is not appropriate based on the data presented, BioCor, through its review committee, could issue a set of general good practice guidelines. The licence, through a regulatory agency, or guidelines, through a regulatory agency or BioCor, may stipulate the need for in-field monitoring and follow-up data requirements. If data presented are not sufficient for evaluation, then further data could be requested before a licence or guidelines are issued. If data presented indicate that a product should not be marketed, then a licence would not be issued. If experience demonstrates that licensing control is necessary and in areas where licensing control does not exist, BioCor would make a recommendation to the minister that specific legislation be created in the area and, in the interim, would, through its seven-

member joint regulatory BioCor review committee, issue conditional 'approval' so as to not unnecessarily impede development.

4.3.4 Concept Overview

The concept for a biotechnology coordination office discussed above is similar to that likely to be adopted in the United States in that it has no legal regulatory mandate. Its sole function is to advise and work with industry and the appropriate federal and provincial regulatory agencies to ensure that there is a uniform application of assessment and regulatory control. This uniformity of control would consider both national needs and international experience and practice. If, in the opinion of the BioCor staff, uniform national standards are not being applied, or if national standards are significantly different from international practice, BioCor's role would be to advise and make recommendations to the minister or ministers responsible.

If increased regulatory control is deemed to be necessary at this time, an alternative concept would be to give BioCor, through the appropriate legislation, the ability to issue research, development and open environmental introduction approval licences for those products which are found not to be covered by current regulatory mandate. This licensing function would continue until such time as the appopriate legislation giving an existing regulatory body jurisdiction was enacted, or until it became apparent that regulatory control was unnecessary.

It is the authors' opinion that the need for a legislated licensing function has not been demonstrated at this time.

5.0 CONCLUSIONS

The environmental regulatory system in Canada and elsewhere has been built up over the last several decades primarily to manage radioactive, chemical and agricultural wastes and products. The regulatory structure and systems of testing and licensing protocols developed for these materials have been reasonably successful. However, because of the potential for persistence and multiplication of biologicals produced using new technologies, and the lack of historical experience with the rapid introduction of distinctly new types of products, changes to regulatory requirements and structures may be needed.

The present lack of formal regulatory control at the research stage reflects a current chemical orientation, where dilution and/or degradation of contaminants will usually ameliorate the effects of small releases. There is perhaps a stronger argument for formal control of small quantities of biologicals which, if released to the environment, could become established and multiply.

Canadian experience with toxic chemicals and radionuclides has shown that there are clearly defined concerns which can be addressed by specific regulatory controls. In the area of biotechnology and, more specifically, rDNA technology, one can hypothesize a series of potential dangers, but specific regulatory controls are difficult to formulate for hypothetical risks. Flexible voluntary or regulatory approaches, which involve guidelines, licensing or certification, while permitting evolution of risk assessment criteria based on accumulating experience, are probably more appropriate.

A regulatory structure which provides effective coordination among the federal and provincial regulatory agencies in concert with international standards will facilitate development of consistent approaches and criteria. This structure could take the form of a new coordinataing office with limited authority complementary to that of existing agencies (Section 4.2.3), or an interagency committee without authority of its own (Section 4.2.2). In either case, it could serve monitoring, data compilation and/or, possibly in concert with or independent of a regulatory authority, licensing or certification functions. The level of authority vested in the coordinating office should depend upon the ability of present legislation and authority to deal with the biotechnology applications of the near future.

As applications for the production and open environmental application of products of the new biotechnologies are presented to the key regulatory agencies, the ability of the present system to deal with these environmental applications, the nature of any problems encountered with existing legislation or authority, and the most appropriate mechanisms of interagency coordination will emerge. This process, however, is expected to take several years, and has the potential, if allowed to develop on an ad hoc basis, of creating a high level of uncertainty as to how the regulatory process works in some areas of open environment application of new biotechnologies.

6.0 RECOMMENDATIONS

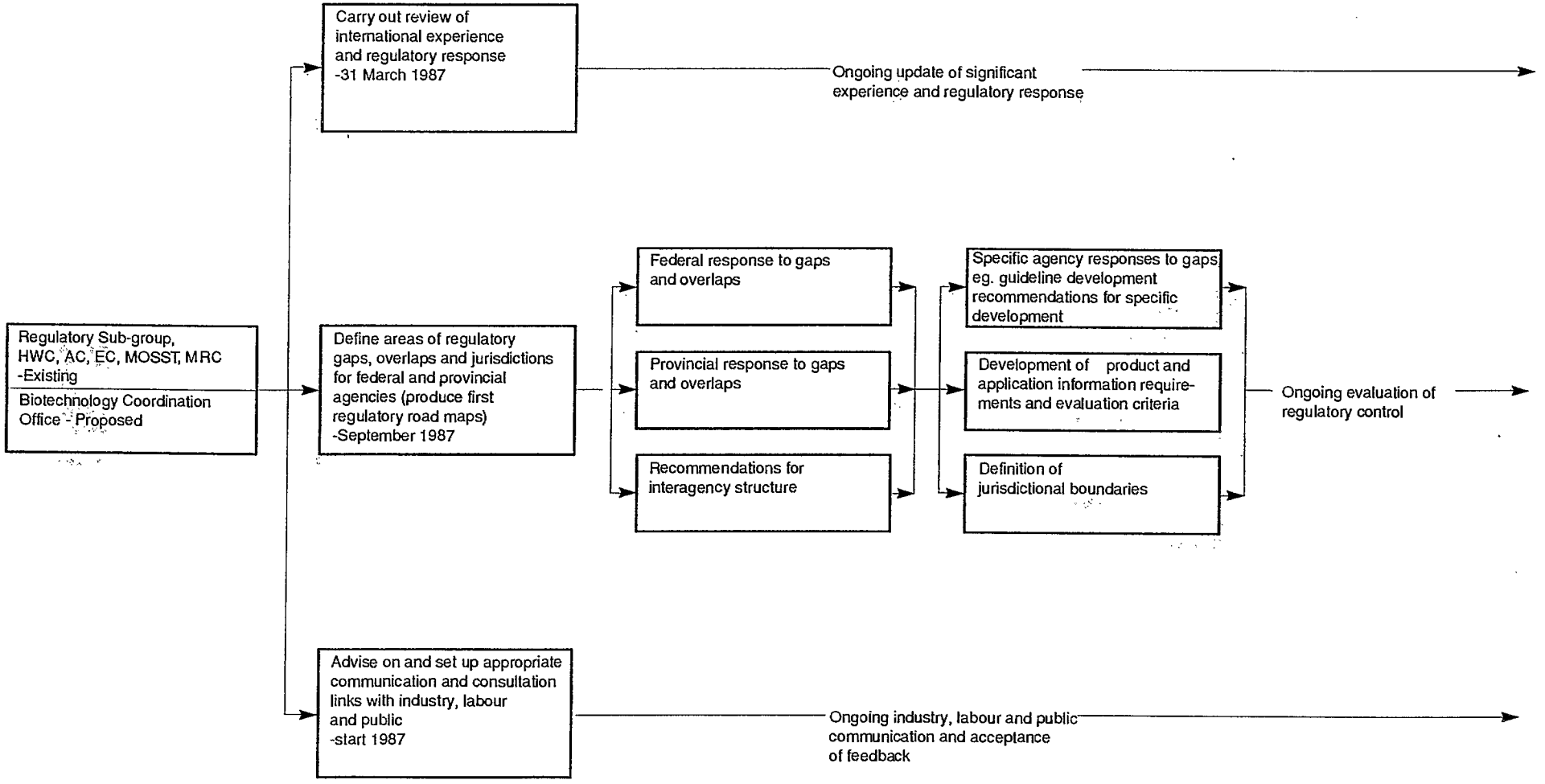
With the objective of accelerating the resolution of jurisdictional problems and demonstrating to the emerging Canadian biotechnology industry and public that Canada has an efficient and appropriate procedure for the regulatory coordination of the emerging new biotechnologies, the following recommendations are made.

1. **Coordination Office:** A Biotechnology Coordination Office should be created in order to coordinate the development of a federal-provincial regulatory system that is responsive to the needs of both public and environmental protection, and at the same time to ensure a Canadian regulatory climate conducive to attracting and retaining a strong biotechnology industry.
2. **Program Plan:** As a first task, this office must produce a program plan which would detail the tasks, and the budget and schedule for each of the tasks necessary to be undertaken in order to achieve the objective of ensuring an appropriate regulatory climate exists within Canada for the biotechnology industry at all levels within the next two to three years.
3. **Participation:** The program plan should ensure that, in developing the regulatory climate, both provincial, industry and public interest group participation is invited, and that specific opportunities are made available to the provinces and industry to participate in the process.
4. **International Coordination:** One of the first tasks the office should undertake is the setting up of a mechanism whereby the production using new biotechnologies of products and their environmental application in other countries can be monitored, along with the regulatory response of the appropriate national regulatory authority. It is suggested that this be done by carrying out a review of the experience and regulatory response that has occurred to date in other countries, and through the setting up of appropriate contact points in other countries to keep abreast of current developments.
5. **Regulatory Road Map:** One possible mechanism of focusing on how the interrelated federal and provincial regulatory jurisdictions may apply to the emerging biotechnology industry and to also produce a useful product for industry and the public would be through the production of a regulatory road map or guide. The focusing mechanism for the production of this guide would be to work through the federal and provincial regulatory system a series of prospective case

studies which would serve to illustrate policy issues as well as the nature of specific testing and risk assessment criteria.

One possible way to visualize the recommended program plan, which allows proceeding from where we are now to where we wish to be within two to three years, along with an initial proposed schedule, is shown in Figure 6.1.

FIGURE 6.1 Biotechnology Safety and Regulation Program Plan Outline



7.0 REFERENCES

A.D. Little, Inc. 1984. Study of Federal Biotechnology Policy Issues. Report prepared for the Division of Policy Research and Analysis, National Science Foundation, Washington, D.C. NSF/PRA-84008.

Alexander, M. 1981. Why microbial predators and parasites do not eliminate the prey and hosts. Ann. Rev. Microbiol. 35: 113-133.

Alexander, M. 1985. Spread of organisms with novel genotypes. In: Biotechnology and the Environment, Risk and Regulation. A.H. Teich, M.A. Levin and J.H. Pace (Eds.). American Association for the Advancement of Science, Washington, DC.

Ames, B.N., J. McCann and E. Yanasaki. 1975. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mut. Res. 31: 347-364.

Anderson, J.D. and C.S. Cox. 1967. Microbial survival. In: Airborn Microbes. P.H. Gregory and J.C. Monteith (Eds.). Symp. Soc. Gen. Microbiol. 17: 203-226.

APHA. 1975. Standard methods for the examination of water and wastewater. 14th Ed. American Public Health Association, Washington, D.C.

ASTM. 1980. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. Annual Book of ASTM Standards, E729, Part 46.

Bachrach, H.L. 1982. Recombinant DNA technology for the preparation of subunit vaccines. J. Am. Vet. Med. Assoc. 181: 992-999.

Barnthouse, L.W. and A.V. Palumbo. 1985. Assessing the transport and fate of bioengineered microorganisms in the environment. CONF-8503163-1.

Barton, K.A. and W.J. Brill. 1983. Prospects in plant genetic engineering. Science 219: 671-676.

Bernstein, L.D. 1982. Monoclonal antibodies: Prospects for cancer treatment. In: Immunological Approaches to Cancer Therapeutics. John Wiley & Sons, New York.

Billiau, A. 1981. Perspectives in cancer research: the clinical value of interferons as antitumor agents. Eur. J. Cancer & Clin. Oncology 17: 949-967.

Blattner, F.R., B.G. Williams, A.E. Blechl, K. Denniston-Thompson, A. Faber, L. Furlong, D.J. Grunwald, D.O. Keifer, D.D. Moore, J.W. Schum, E.L. Sheldon and O. Smithies. 1977. Charon phages: safer derivatives of bacteriophage lambda for DNA cloning. Science 196: 161-169.

Bloom, A.L. 1983. Benefits of cloning genes for clotting factors. Nature 303: 474-475.

Bovallius, A., B. Bucht, R. Roffey and E. Henningson. 1978. Long-range air transmission of bacteria. App. Environ. Microbiol. 35: 1231-1232.

Bovallius, A., R. Roffey and E. Henningson. 1980. Long-range transmission of bacteria. Ann. N.Y. Acad. Sci. 353: 186-200.

Brierley, C.L. 1978. Bacterial leaching. CRC Critical Reviews in Microbiology 6: 207-262.

Brierley, C.L. 1982. Microbiological mining. Scientific American, pp. 44-53.

Brill, W.J. 1985. Safety concerns and genetic engineering in agriculture. Science 227: 381.

Buchanan, R.E. and N.E. Gibbons (Eds.). 1974. Bergey's Manual of Determinative Bacteriology. Eighth Edition, The Williams and Wilkins Co., Baltimore, MD.

Bungay, H.R. 1981. The Biomass Options. John Wiley & Sons, New York.

Campbell, W.C., M.H. Fisher, E.O. Stapley, et al. 1983. Ivermectin: a potent new antiparasitic agent. Science 221: 823-828.

Chakrabarty, A.M. 1978. Genetic mechanisms in metal-microbe interactions. In: Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena. L.E. Murr et al. (Eds.). Academic Press, New York.

Choi, Y.J. and D.E. Tribe. 1982. Continuous production of phenylalanine using an Escherichia coli regulatory mutant. Biotech. Letters 4: 223-228.

Choo, K.H., K.G. Gould, D.J.G. Rees and G.G. Brownlee. 1982. Molecular cloning of the gene for human anti-haemophilic factor IX. Nature 299: 178-180.

Cochrane, A.H., F. Santoro, V. Nussenzwei et. al. 1982. Monoclonal antibodies identify the protective antigens of sporozoites of Plasmodium knowlesi. Prod. Natl. Acad. Sci. 79: 5651-5655.

Colwell, R.K., Norse, E.A., Pimentel, D., F.E. Sharples and D. Simberloff. 1985. Genetic engineering in agriculture. Science 229: 111-112.

Cooney, C.L. 1983. Prospects for chemicals and fuels production by fermentation. In: Basic Biology of New Developments in Biotechnology. Plenum Press, New York.

Cooper, T. 1985. The impact of biotechnology on the pharmaceutical industry. In: Biotechnology and the Environment, Risk and Regulation. A.H. Teich, M.A. Levin and J.H. Pace (Eds.). American Association for the Advancement of Science, Washington, DC.

Crawford, M. 1986a. Larger public sector role sought on biotech. Science 232: 15-16.

Crawford, M. 1986b. Fuqua biotech bill sets stage for industry debate. Science 231: 1501.

Culliton, B.J. 1985. New biotech review board planned. Science 229: 736-737.

Daniel, J.R. and R.L. Whistler. 1981. Industrial chemicals from cereals. In: Cereals: A Renewable Resource. American Association of Cereal Chemists, St. Paul, Minn.

Datta, N. 1979. Plasmid classification: incompatibility grouping. In: Plasmids of Medical, Environmental, and Commercial Importance. K.A. Timmis and A. Puhler (Eds.). Elsevier/North-Holland, Amsterdam, pp. 3-12.

Day, P.R. 1983. Long-term goals in agricultural plant improvement. In: Genetic Manipulation: Impact on Man and Society. W. Arber, K. Illmensee, W.J. Peacock, et al. (Eds.). Cambridge University Press, Cambridge, England.

Della-Porta, A.J. 1983. Current status of foot-and-mouth disease vaccines including the use of genetic engineering. Australian Veterinary Journal 60: 129-135.

Demain, A.L. 1981. Industrial microbiology. Science 214: 987-995.

Dickson, D. 1986. Gene-splicing debate heats up in Germany. Science 232: 13-14.

Dimmick, R.L. and A.B. Akers. 1969. An Introduction to Experimental Aerobiology. Wiley-Interscience, New York.

Dixon, B. 1985. Genes: out of the laboratory, into the unknown. New Scientist, pp. 44-49.

Dougan, G., J.H. Crosa and S. Falkow. 1978. Mobilization of the Escherichia coli plasmid ColE1 (colicin E1) and ColE1 vectors used in recombinant DNA experiments. J. Infect. Diseases 137: 676-680.

Emert, G.H. and R. Katzen. 1981. Chemicals from biomass by improved enzyme technology. In: Biomass as a Nonfossil Fuel Source. D.L. Klass (Ed.). Symposium Series 144, American Chemical Society, Washington, D.C.

Enei, H., K. Sato, Y. Anzai, et al. 1975. Fermentative production of riboflavin. U.S. Patent No. 3,900,368.

EPA. 1982. Daphnia acute toxicity test. EG-1. U.S. Environmental Protection Agency.

EPA. 1984. Proposed Policy Regarding Certain Microbial Products. Federal Register 49(252): Part II, Dec. 31.

EPA. 1986. Assessing EPA's Biotechnology Research and Information Needs. Report of the Study Group on Biotechnology. U.S. Environmental Protection Agency, Science Advisory Board, Washington, D.C.

EPS. 1982. Standard procedure for testing the acute lethality of liquid effluents. Environmental Protection Service, Ottawa.

Erikisson, E. 1963. The yearly circulation of sulfur in nature. J. Geophys. Res. 68: 4001-4008.

Evans, M.R. and J.D. Owens. 1973. Soil bacteria in land-drainage water. Water Research 7: 1295-1300.

Eveleigh, D.E. 1981. The microbiological production of industrial chemicals. Scientific American 245: 155-178.

Federal Register. 1985. Coordinated Framework for Regulation of Biotechnology; Establishment of the Biotechnology Science Coordinating Committee; Notice. Federal Register 50(220): Part III, Nov. 14.

Fisher, E. 1985. The management and assessment of risks from recombinant organisms. Journal of Hazardous Materials 10: 241-261.

Fox, J.L. 1984. Rifkin takes aim at USDA animal research. Science 226: 321.

Fox, A.M., J.S. Sciortino and D.C. Smith, III. 1983. International Health and Safety Regulation of Biotechnology, a comparison between the European communities, the United Kingdom, France, the Federal Republic of Germany, Switzerland and Japan and the United States. Report prepared by Kage, Scholer, Fierman, Hays and Handler for the U.S. Congress, Office of Technology Assessment, Washington, D.C.

Gatz, R.L., B.A. Young, TJ. Facklam and D.A. Scantland. 1983. Monoclonal antibodies: emerging product concepts for agriculture and food. Biotechnology, pp. 337-341.

Genex Corp. 1983. Impact of biotechnology on the specialty chemicals industry. Contract paper prepared for the Office of Technology Assessment, U.S. Congress.

Gillett, J.W., S.A. Levin, M.A. Harwell, M. Alexander, D.A. Andow and A.M. Stern. 1984. Potential impacts of environmental release of biotechnology products: assessment, regulations, and research needs. Ecosystems Research Center, ERC-075.

Gilliland, D., Z. Gary, R.J. Steplweski, et al. 1980. Antibody-directed cytotoxic agents: use of monoclonal antibody to direct the action of toxin A chains to colorectal carcinoma cells. Proc. Natl. Acad. Sci. 77: 4539-4543.

Goldman, S., Y. Shabtal, C. Rubinovitz, et al. 1982. Emulsan in Acinetobacter calcoaceticus RAG-1: distribution of cell-free and cell-associated cross-reacting material. Appl. Environ. Microbiol. 44: 165-170.

Graham, D., C. Quinn and L. Bradley. 1977. Quantitative studies on the generation of gerosols of Erwinia carotovora var. atrosptica by simulated raindrop impaction on blackleg-infected potato stems. J. Applied Bacteriology 43: 413-424.

Greenway, J.C., Jr. 1967. Extinct and Vanishing Birds of the World. Dover, New York.

Guiney, D.G., Jr. and C.E. Davis. 1978. Identification of a conjugative R plasmid in Bacteroides ochraceus capable of transfer to Escherichia coli. Nature 274: 181-182.

Haber, C.L., L.N. Allen, S. Zhao and R.S. Hanson. 1983. Methylotrophic bacteria: biochemical diversity and genetics. Science 221: 1147-1153.

Hardy, R.W.F. 1985. Biotechnology in agriculture; status, potential, concerns. In: Biotechnology and the Environment, Risk and Regulation. A.H. Teich, M.A. Levin and J.H. Pace (Eds.). American Association for the Advancement of Science, Washington, DC.

Harris, T.J.R., P.A. Lowe, A. Lyons, et al. 1982. Molecular cloning and nucleotide sequence of cDNA coding for preprochymosin. Nucleic Acids Research 10: 2177-2187.

Harrison, K. and D. Hattis. 1985. Containment of genetically-engineered microorganisms: a comparison of expected releases during greenhouse trials with releases in ordinary research and development. MIT, Centre for Technology, Policy and Industrial Development, Cambridge. CTPID 85-2.

Harsanyi, Z. 1985. Biotechnology and the environment. In: Biotechnology and the Environment, Risk and Regulation. A.H. Teich, M.A. Levin and J.H. Pace (Eds.). American Association for the Advancement of Science, Washington, DC.

Harwood, C.R. 1980. Plasmids. In: Microbiological Classification and Identification. M. Goodfellow and R.G. Board (Eds.). Academic press, New York.

Henley, D.C. 1986. Coordinated study on government processes in safety and regulation of modern biotechnology. Draft Report to the Interdepartmental Committee on Biotechnology. Environmental Protection Service, Ottawa.

Hill, W.E., W.L. Payne, R.J. Crouch, et al. 1984. Genetic methods for the detection of microbial pathogens. Identification of enterotoxigenic E. coli by DNA colony hybridization: collaborative study. J. Assoc. Off. Anal. Chem. 67: 801-807.

Hutchinson, G.E. 1957. A Treatise on Limnology, Vol. I. Geography, Physics and Chemistry. Wiley, N.Y.

ICPEMC. 1983. Screening Strategy for Chemicals that are Potential Germ-cell Mutagens in Mammals. Committee 1 Final Report. International Commission for Protection Against Environmental Mutagens and Carcinogens. Elsevier Medical Press, Amsterdam.

Itoh, T. 1985. Japan: release a long way off. Bio/Technology 3: 1072.

Jarman, I.R. 1979. Bacterial alginate synthesis. In: Microbial Arginate Synthesis. RC.W. Berkeley, G.W. Gooday and D.C. Ellwood (Eds.). Academic Press, New York.

Jaroff, L. 1986. Fighting the biotech wars. Time 127(16): 60-62.

Jeans, A. 1977. Dextrans and pullulans: industrially significant alpha-D-glucans. In: Extracellular Microbial Polysaccharides. P.A. Sanford and A. Laskin (Eds.). American Chemical Society, Washington, D.C.

John, H., J. Nijkamp and E. Veltkamp. 1981. Genetic organization and expression of non-conjugative plasmids. In: Molecular Biology, Pathogenicity and Ecology of Bacterial Plasmids. S. Levy, R.C. Clowes and E.L. Koenig (Eds.). Plenum Press, New York.

Jones, B. and J. Cookson. 1983. Natural atmospheric microbial conditions in a typical suburban area. App. Env. Microbiology 45: 919-934.

Karns, J.S., J.J. Kilbane, D.K. Chatterjee and A.M. Chakrabarty. 1984. Microbial biodegradation of 2,4,5-trichlorophenoxyacetic acid and chlorophenols. Basic Life Sci. 28: 3-21.

Kilbane, J.J., D.K. Chatterjee, J.B. Karns, et al. 1982. Biodegradation of 2,4,5-trichlorophenoxyacetic acid by a pure culture of Pseudomonas cepacia. Appl. Envir. Microbiol. 44: 72-78.

King, S.R. 1985. Economic impacts of biotechnology. In: Biotechnology and the Environment, Risk and Regulation. A.H. Teich, M.A. Levin and J.H. Pace (Eds.). American Association for the Advancement of Science, Washington, DC.

Kleid, D.G., D. Yansura, B. Small, et al. 1981. Cloned viral protein vaccine for foot-and-mouth disease responses in cattle and swine. Science 214: 1125-1129.

Krolick, K.A., C. Villemex, P. Isakson, et al. 1980. Selective killing of normal or neoplastic B cells by antibodies coupled to the A chain of ricin. Proc. Natl. Acad. Sci. 77: 5419.

Langone, J. 1983. Monoclonals: the super antibodies. Discover, 68-72.

Law, R.M., J. Adelman, S.C. Block, et al. 1981. The sequence of human serum albumin cDNA and its expression in E. coli. Nucleic Acids Research 9: 6103-6127.

Leemans, J., C. Shaw, R. DeBlaere, et al. 1981. Site-specific mutagenesis of agrobacterium Ti plasmids and transfer of genes to plant cells. J. Molec. Appl. Genet. 1: 149-164.

Levin, B.R., F.M. Stewart and V.A. Rice. 1979. The kinetics of conjugative plasmid transmission: fit of a simple mass action model. Plasmid 2: 247-260.

Levin, M.A., G.H. Kidd, R.H. Zaugg and J.R. Swatz. 1983. Applied Genetic Engineering: Future Trends and Problems. Noyes Data Corporation, New Jersey.

Liang, L.N., J.L. Sinclair, L.M. Mallory and M. Alexander. 1982. Fate in model ecosystems of microbial species of potential use in genetic engineering. Applied and Env. Microbiology 44: 709.

Lighthart, B. 1984. Microbial aerosols: estimated contribution of combine harvesting to an airshed. Appl. Environ. Microbiol. 47: 430-432.

Lighthart, B., J. Spendlove and T. Akers. 1979. Sources and characteristics of airborne materials. Bacteria and viruses. In: Aerobiology: The Ecological Approach. Dowden, Hutchinson and Ross, Stroudsburg, PA, pp. 11-22.

Lipinsky, E.S. 1981. Chemicals from biomass: petrochemical substitution options. Science 212: 1465-1471.

Litchfield, J.H. 1983. Single-cell proteins. Science: 219: 740-746.

Maugh, T.H. 1978. Chemicals: how many are there? Science 199: 162.

Mbikay, M., R. Brzezinski and J. Thirion. 1983. Differential expression of cloned mouse metallothionein sequences in Escherichia coli. DNA 2(1): 23-29.

McCormick, D. 1985. No escaping free release. Bio/Technology 3: 1065-1067.

McCoy, E.L. and C. Hagedorn. 1979. Quantitatively tracing bacterial transport in saturated soil systems. Water, Air and Soil Pollution 11: 467-479.

McGaughey, W.H. 1985. Insect resistance to the biological insecticide Bacillus thuringiensis. Science 229: 193-195.

McMichael, A.J. and J.W. Fabre. 1982. Monoclonal Antibodies in Clinical Medicine. Academic Press, New York.

Melchers, F., M. Potter and N.L. Warner (Eds.). 1978. Lymphocyte Hybridomas. Curr. Top. Microbiol. Immunol, Vol. 81. Springer-Verlag, New York.

Miller, J. 1986. Canada: building a regulatory framework. Bio/Technology, Vol. 4: 206-207.

MRC. 1977. Guidelines for the Handling of Recombinant DNA Molecules and Animal Viruses and Cells. Medical Research Council, Ottawa.

MRC. 1980. Guidelines for the Handling of Recombinant DNA Molecules and Animal Viruses and Cells. Medical Research Council, Ottawa.

Miller, J.A. 1983. Microbial antifreeze: gene splicing takes to the field. Science News 124: 132.

Milstein, C. 1980. Monoclonal antibodies. Scientific American 243: 66-74.

MOE. 1983. Protocol to determine the acute lethality of liquid effluents to fish. Ministry of the Environment, Water Resources Branch, Rexdale.

Montenecourt, B.S. 1983. Application of recombinant DNA technology to cellulose hydrolysis. Biotechnology 1: 166.

Murasugi, A. and R.B. Wallace. 1984. Biotin-labelled oligonucleotides: enzymatic synthesis and use as hybridization probes. DNA 3: 269-277.

NBAC. 1984. National Biotechnology Advisory Committee. Annual Report. Ministry of State for Science and Technology, Ottawa.

Newman, S. 1985. Dynamic balance in living systems. Lab Notes, pp. 12-13.

Newmark, P. 1983. What chance of a malaria vaccine? Nature 302: 473.

Ng, T.K., R.M. Busche, C.C. McDonald, et al. 1983. Production of feedstock chemicals. Science 219: 733-740.

Nowinski, R.C., M.R. Tam, L.C. Golstein, et al. 1983. Monoclonal antibodies for diagnosis of infectious diseases in humans. Science 219: 637-643.

OECD. 1981. Daphnia sp. 14-day reproduction test (including an acute immobilization test). Guidelines for Testing Chemicals, 202. Organization for Economic Cooperation and Development.

OECD. 1986. Recombinant DNA safety considerations. Safety considerations for industrial, agricultural and environmental applications of organisms derived by recombinant DNA techniques. Organization for Econommic Cooperation and Development, Report DSTI 8727S E.312.

Orkin, S.H., P.F.R. Little, H.H. Kazazian and C.D. Boehm. 1982. Improved detection of sickle mutation by DNA analysis. N. Eng. J. Med. 307: 32-36.

OTA. 1979. Drugs in livestock feed. OTA-F-91, U.S. Congress, Office of Technology Assessment, Washington, D.C.

OTA. 1981. Impacts of applied genetics: micro-organisms, plants, and animals. OTA-HR-132, U.S. Congress, Office of Technology Assessment, Washington, D.C.

OTA. 1984. Commercial biotechnology, an international analysis. U.S. Congress, Office of Technology Assessment, OTA-BA-218.

OTAF. 1982. Patent Profiles: Biotechnology. U.S. Department of Commerce, Patent and Trademark Office, Office of Technology Assessment and Forecast, Washington, D.C.

Page, G.S., K. Harrison and D. Hattis. 1985. Industrial Innovation Based on Undirected Mutagenesis of Microorganisms - Implications for Regulation under TSCA. Centre for Technology, Policy and Industrial Development, Report #CTPID 85-1.

Palmiter, R.D., R.L. Brinster, R.E. Hammer, et al. 1982. Dramatic growth of mice that develop from eggs microinjected with metallothionein - growth hormone fusion genes. Nature 300: 611-615.

Peel, C.J., D.E. Bauman, R.C. Gorewit, et al. 1981. Effect of exogenous growth hormone on lactational performance in high yielding dairy cows. J. Nutr. 111: 1662-1671.

Preston, R.J., A.W. Bender, J.G. Brewen, A.V. Carrano, J.A. Heddle, A.F. McFee, S. Wolff and J.S. Wassom. 1981. Mammalian in vivo and in vitro cytogenetic assays. A report to the U.S. EPA Gene-tox program. Mut. Res. 87: 143-188.

Pritchard, P.H. and A.W. Bourquin. 1984. The use of microcosms for evaluation of interactions between pollutants and microorganisms. Adv. Microbial Ecol. 7: 133-217.

Ralph, B.J. 1982. The implications of genetic engineering for biologically assisted hydrometallurgical processes. In: Genetic Engineering Commercial Opportunities in Australia. Australian Government Publishing Service, Canberra.

Reanney, D. 1976. Extrachromosomal elements. Bacteriological Reviews, Sept. 1976, pp. 552-590.

Rhein, R. 1985. The battle for authority over biotech research. Chemical Week, pp. 24-25.

Roberts, R.J. 1976. Restriction endonucleases. CRC Critical Reviews of Biochemistry 4: 123-164.

Sanderson, K.E. 1976. Genetic relatedness in the family Enterobacteriaceae. Ann. Rev. Microbiol. 30: 327-349.

Searle, A.G. 1975. The specific locus test in the mouse. Mut. Res. 31: 277-290.

Sharples, F.E. 1982. Spread of organisms with novel genotypes: thoughts from an ecological perspective. Oak Ridge National Laboratory, Environmental Sciences Division, Pub. No. 2040. ORNL/TM-8473.

Sharples, F.E. 1983. Spread of organisms with novel genotypes: thoughts from an ecological perspective. Rec. DNA Tech. Bul. 6: 43-56.

Simberloff, D. Community effects of introduced species. In: Biotic Crises in Ecological and Evolutionary Time. M.H. Nitecki (Ed.). Academic Press Inc.

Skotnicki, M.L., K.J. Lee, D.E. Tribe, et al. 1982. Genetic alteration of Zymomonas mobilis for ethanol production. In: Genetic Engineering of Microorganisms for Chemicals. A. Hollaender (Ed.). Plenum Press, New York.

Sonea, S. and M. Panisset. 1980. A New Bacteriology. Jones and Bartlett, Boston, Mass.

Stackbrandt, E. and C.R. Woese. 1984. The phylogeny of prokaryotes. Microbiological Sciences 1: 117-122.

Stanier, R., M. Douderoff and E.A. Adelberg. 1976. The Microbial World. Prentice-Hall, Inc., New Jersey.

Stent, G.S. Molecular Genetics. W.H. Freeman and Co., San Francisco.

Strauss, H., D. Hattis, G.S. Page, K. Harrison, S.R. Vogel and C.C. Caldart. 1985. Direct release of genetically-engineered microorganisms: a preliminary framework for risk evaluation under TSCA. MIT, Centre for Technology, Policy and Industrial Development, Cambridge. CTPID 85-3.

Sun, M. 1985. EPA approves field test of altered microbes. Science 230: 1015-1016.

Sun, M. 1986a. Monsanto opens files on genetic release test. Science 231: 1065.

Sun, M. 1986b. Local opposition halts biotechnology test. Science 231: 667-668.

Sun, M. 1986c. EPA suspends biotech permit. Science 232: 15.

Suter, G.W., II. 1985. Application of environmental risk analysis to engineered organisms. CONF-8506179-1.

Thorne, C.B. 1974. Transduction. In: Handbook of Microbiology, Vol. IV. A. Laskin and H. Lechevalier (Eds.). CRC Press, Cleveland, Ohio, pp. 517-521.

Totten, P.A., K.K. Holmes, H.H. Handsfield, J.S. Knapp, P.L. Perine and S. Falkow. 1983. DNA hybridization technique for the detection of Neisseria gonorrhoeae in men with urethritis. J. Infect. Diseases 148: 462-471.

Tsung, Y., A. Milkunsky and E. Alpert. 1980. Derivation and characterization of a monoclonal hybridoma antibody specific for human alpha-fetoprotein. J. Immunol. Meth. 39: 363-368.

U.S. FDA. 1984. Statement of Policy for Regulating Biotechnology Products. Federal Register 49(252): Part II, Dec. 31.

Vogel, C.W. and H.J. Muller-Eberhard. 1981. Induction of immune cytolysis: tumor-cell killing by complement is initiated by covalent complex of monoclonal antibody and stable C3/C5 convertase. Proc. Natl. Acad. Sci. 78: 7707-7711.

Volger, H.C. 1981. Microbial leaching. In: Biotechnology: A Dutch Perspective. J.H.F. van Apeldoorn (Ed.). Delft University Press, Delft, Netherlands.

Vournakis, J.N. and R.P. Elander. 1983. Genetic manipulation of antibiotic producing microorganisms. Science 219: 703-709.

Walgate, R. 1985. Europe: a few cooks too many? Bio/Technology, Vol. 3: 1070-1071.

Wands, J.R., R.I. Carlson, H. Schoemaker, et al. 1981. Immunodiagnosis of hepatitis B with high affinity IgM monoclonal antibodies. Proc. Natl. Acad. Sci. 78: 1214-1218.

Waterworth, D.G. 1981. Single cell protein. Outlook on Agriculture 10: 403-408.

Wells, J. 1977. Extracellular microbial polysaccharides - a critical review. In: Extracellular Microbial Polysaccharides. P.A. Sanford and A. Laskin (Eds.). American Chemical Society, Washington, D.C.

White, D.H., G.A. Smith, J.H. Gehron, J.H. Parker, R.H. Findlay, R.F. Martz and H.L. Frederickson. 1983. The groundwater aquifer microbiota: biomass, community structure, and nutritional status. In: Developments in Industrial Microbiology, Vol. 24. C.H. Nash and L.A. Underkofler (Eds.). Society for Industrial Microbiology, Arlington, VA, pp. 201-213.

WHO. 1984. Health Impact of Biotechnology. Report on a working group, Dublin, 9-12 November 1982. World Health Organization, Regional Office for Europe, Copenhagen.

Witt, J.M. and J.W. Gillet. 1977. Terrestrial Microcosms and Environmental Chemistry. Proceedings of Symposium, Corvallis, Oregon. National Science Foundation, NSF/RA 79-0026, pp. 147.

Yelton, D.E. and M.D. Scharff. 1980. Monoclonal antibodies. Amer. Scientist 68: 510.

Zimmer, S.J. 1982. The impacts of applied genetics in animal agriculture. Contract report prepared for the Office of Technology Assessment, U.S. Congress.

APPENDIX 1

Persons Contacted and From Whom Ideas Were Obtained During the Preparation of This Document

PERSONS CONTACTED AND FROM WHOM IDEAS WERE OBTAINED DURING THE PREPARATION OF THIS DOCUMENT

Penny Chan	Ontario Ministry of Labour
Leslie Whitby	Environment Canada
Doreen Henley	Environment Canada
Richard Letilley	Saskatchewan Science and Technology
John Cross	Philombios, Saskatoon
Warren Steck	NRC Plant Biotechnology Institute, Saskatoon
Ivy Wyle	Ontario Ministry of the Environment
Goeff Jenkins	Ontario Ministry of the Environment
Ann Robinson	Ontario Ministry of Labour
Alan Cairnie	NRC Associate Committee
D. Shindler, J. Ferguson	Ministry of State for Science and Technology
John Evans	Allelix, Mississauga
Niel Gray	CIL, Mississauga
Bernie Glick	University of Waterloo
Cecil Fosberg	University of Guelph
Duncan Currie	Alberta Research Council
Dan Billing	Science Institute of the N.W.T.
George Greenland	Development Services, Government of Newfoundland and Labrador
Andris Rode	Executive Council Office, Yukon Territory
Klaus Hellenbrand	Nova Scotia Research Foundation
Dale Turner	P.E.I. Development Agency
Deborah George	Ministry of Industry, Trade and Investment
Georges Lagace	Service de science et procedes biologiques Quebec
Ron Humble	Manitoba Industry, Trade and Technology
Daniel Clarke	Department of Commerce and Development, N.B.
Alex Guy	Saskatchewan Science and Technology
Martin Walmsly	Ontario Ministry of Industry and Trade
Peter Larkin	University of British Columbia
Terry Mailloux	Pacific Isotopes and Pharmaceuticals, Vancouver
Charles Visner	Research Products Council, N.B.
Michael Gray	Dalhousie University, N.S.