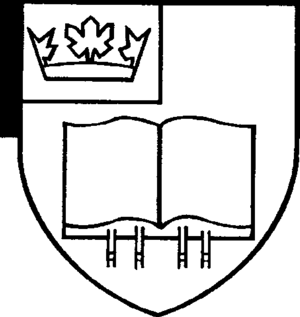
Elements of Precaution: Recommendations for the Regulation of Food Biotechnology in Canada

An Expert Panel Report
on the Future of Food Biotechnology
prepared by

The Royal Society of Canada

at the request of
Health Canada
Canadian Food InspectionAgency
and
Environment Canada



"studiis eodem diversis nitimur"
"different paths, one vision"

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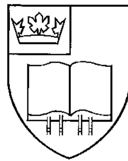

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EXPERT PANEL ON THE FUTURE OF FOOD BIOTECHNOLOGY

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The opinions expressed in this report are those of the authors and do not necessarily represent those of the Royal Society of Canada or the opinion or policy of Health Canada, the Canadian Food Inspection Agency and Environment Canada.

William Leiss, President
The Royal Society of Canada

January 30, 2001

Dear Dr. Leiss:

We are pleased to enclose a copy of the report *Elements of Precaution: Recommendations for the Regulation of Food Biotechnology in Canada*. The report has been prepared by the Expert Panel on the Future of Food Biotechnology, established by the Committee on Expert Panels of the Royal Society of Canada, in response to a request from Health Canada, the Canadian Food Inspection Agency, and Environment Canada. Our report addresses the questions posed by these agencies in the Terms of Reference negotiated between the government agencies and the Expert Panel, which are outlined in the Executive Summary and the Introduction of the enclosed report. These Terms of Reference asked the Expert Panel to provide advice on the Canadian regulatory system and the scientific capacity the federal government requires into the 21st century to ensure the safety of new food products being developed through biotechnology.

The enclosed report responds to this request by summarizing the scientific developments that have led to the current status of application of the technology and identifying the social and scientific dynamics that foreshadow new applications of biotechnology. It examines in detail the safety implications of these applications for human and animal health and the natural environment. The report also critically examines the current standard principles and practices governing the regulation of food biotechnology both in Canada and internationally, and makes a series of recommendations in three areas: 1) those concerning fundamental policies and principles governing the regulation of biotechnology, 2) those concerning specific Canadian regulations and guidelines, and 3) those concerning the regulatory process itself.

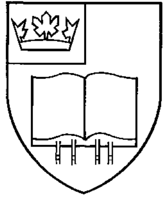
We are happy to report that the enclosed report represents a broad agreement among the members of the Panel. This is not to imply that every member would express all the arguments and conclusions in exactly the same way, or that some members would not favour additional or even stronger recommendations in some areas. Despite the broad agreement among the members, the Expert Panel has agreed that every member should be free to express his or her own individual interpretations and points of difference freely. It is a tribute to the RSC Committee on Expert Panels that a panel of this size and diversity of expertise and opinion was able to work in such a highly collegial and collaborative manner. Many of the issues with which we were charged are complex and controversial. They generated vigorous debate within the Panel. We would like to thank all the members of the Panel for the energy and time they devoted to the completion of this report in such a collaborative spirit.

This is a large and complex topic. The Panel had to carry out its task in a relatively short time span given the enormity of the task. We were able obtain the information we needed and to meet the deadlines we faced largely due to the able support provided to the Panel by Dr. Geoffrey Flynn, Chair of the Committee on Expert Panels, and Ms. Sandy Jackson, the Project Administrator for the Royal Society of Canada. We are deeply indebted to them for their tireless assistance.

We hope that Health Canada, the Canadian Food Inspection Agency, and Environment Canada will find our recommendations useful in the important task they face in the regulation of food biotechnology in Canada.

Yours sincerely,

Conrad Brunk and Brian Ellis
Co-Chairs, RSC Expert Panel on the Future of Food Biotechnology



The Royal Society of Canada

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Prefatory Note

In November, 1999 Health Canada's Health Products and Foods Branch approached the Royal Society of Canada with a request to commission an Expert Panel to provide advice to ensure the safety of new food products being developed through biotechnology. The Society agreed to do so, and the Committee on Expert Panels undertook the task of screening and selecting the individuals whose names now appear as the authors of this report for panel service.

The report entitled *Elements of Precaution: Recommendations for the Regulation of Food Biotechnology in Canada* represents a consensus of the views of all of the Panelists whose names appear on the title page. The Committee wishes to thank the Panel Members and Panel Chairs, the Peer Reviewers, and the Panel staff for completing this very important report within a short period of time.

The Society has a formal and published set of procedures, adopted in October 1996, which sets out how Expert Panel processes are conducted, including the process of selecting Panelists. Interested persons may obtain a copy of those procedures from the Society. The Committee on Expert Panels will also respond to specific questions about its procedures and how they were implemented in any particular case.

The Terms of Reference for this Expert Panel are reproduced elsewhere in this report. As set out in our procedures, the terms are first proposed by the study sponsor, in this case Health Canada, the Canadian Food Inspection Agency and Environment Canada, and accepted provisionally by the Committee. After the Panel is appointed, the terms of reference are reviewed jointly by the Panelists and the sponsor; the Panelists must formally indicate their acceptance of a final Terms of Reference before their work can proceed. These are the terms reproduced in this report.

The Panel first submits a draft of its final report in confidence to the Committee, which arranges for another set of experts to do a peer review of the draft. The Peer Reviewer comments are sent to the Panel, and the Committee takes responsibility for ensuring that the Panelists have addressed satisfactorily the Peer Reviewer comments.

The Panel's report is released to the public without any prior review and comment by the study sponsor. This arm's-length relationship with the study sponsor is one of the most important aspects of the Society's Expert Panel process.

Inquiries about the Expert Panel process may be addressed to the Chair, Committee on Expert Panels, Royal Society of Canada.

Dr. Geoffrey Flynn, FRSC
Chair, Committee on Expert Panels

on behalf of the Committee Members for this Panel:

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Christopher Garrett, FRS, FRSC, University of Victoria
Daniel Krewski, University of Ottawa
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January 30, 2001

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EXECUTIVE SUMMARY

This Report is a response to a request to the Royal Society of Canada from Health Canada, the Canadian Food Inspection Agency and Environment Canada that an Expert Panel be assembled to provide advice on a series of questions related to the safety of new food products being developed through the use of new genetic engineering technologies. The Terms of Reference asked the Panel "to provide Health Canada, the Canadian Food Inspection Agency and Environment Canada with advice on our regulatory system and the scientific capacity that the federal government requires into the 21st century to ensure the safety of new food products being developed through biotechnology". We were specifically charged to address the following issues:

To forecast:
- *the types of food products being developed through biotechnology that could be submitted for regulatory safety reviews by Health Canada and/or the Canadian Food Inspection Agency over the next 10 years;*
- *the science likely to be used to develop these products; and*
- *any potential short- or long-term risks to human health, animal health and the environment due to the development, production or use of foods derived from biotechnology.*

To assess approaches and methodologies developed in Canada and internationally to evaluate the safety of foods being developed through biotechnology, including those being developed by the World Health Organization, the Food and Agricultural Organization and the Codex Alimentarius Commission.

To identify:
- *the scientific capacity that will be needed to ensure the safety of new foods derived from biotechnology, including human resources for research, laboratory testing, safety evaluation, and monitoring and enforcement; and*
- *any new policies, guidelines and regulations related to science that may be required for protecting human health, animal health and environmental health.*

This Report addresses these issues in the following way.

Chapter 1 clarifies the Panel's interpretation of its mandate and the Terms of Reference. It attempts to delineate clearly the range of scientific and non-scientific issues that fall within its mandate, those that fall clearly outside it, and those related issues that need to be addressed to

provide comprehensive answers to the questions posed by the mandate. Chapter 1 also summarizes the process by which the Panel produced the Report.

Chapter 2 responds to the mandate to forecast the future directions in the development of agricultural biotechnology. It does so by summarizing the scientific developments that have led to the current status of application of the technology. It identifies the social and scientific dynamics driving its current development, and points to technological developments that are likely to bring new applications of biotechnology. Many of the themes summarized in Chapter 2 are developed in greater detail in subsequent chapters dealing with specific health and environmental risks.

Chapter 3 summarizes the system currently in place for the regulation of agricultural biotechnology in Canada. The chapter recommends implementation of an independent process for auditing of the scientific and ethical aspects of regulatory decision making.

Chapter 4 is the first of three chapters that conduct the scientific identification of the short- and long-term risks the Panel found to be most important for regulatory concern in Canada. It focuses on the direct risks to human health posed by genetically modified (GM) food. Part 1 of Chapter 4 considers the specific problems related to the use of the classical risk assessment methodologies for the assessment of toxicological risks from GM foods, especially the assessment of the safety of whole foods. Part 2 focuses on the critical issues related to the identification of potential allergens in GM foods, and makes recommendations for strengthening the scientific capacity for identifying and assessing the allergenicity of new or unexpected proteins in GM foods. Part 3 points to the need to consider the impacts of genetic engineering modifications on the nutritional value of the resulting food.

Chapter 5 considers the potential direct impacts of genetic engineering upon the health and welfare of agricultural animals, as well as the indirect impacts upon wild animals. Part 1 identifies the risks associated with the genetic modification of fish and farm animals themselves, while Part 2 focuses upon the risks associated with GM feeds, feed additives and metabolic modifiers administered to food-producing animals. Chapter 5 makes a variety of recommendations for the more rigorous assessment of the impacts upon animal health and welfare, genetic diversity and sustainability, as well as upon human consumers of GM animals and animal products.

Chapter 6 identifies what the Panel considered to be the most significant potential risks to various aspects of the natural environment posed by agricultural biotechnology. The chapter is divided into four parts, each dealing with the impacts of potential gene flow upon different sectors of the natural environment — microorganisms and soil microflora, wild and non-GM plants, target and non-target insects, and wild fish. Recommendations following each of these sections identify a series of more refined environmental assessments that need to be added to the Canadian regulatory process to protect more adequately important environmental values.

Chapter 7 introduces a series of three final chapters that deal with critical methodological approaches and assumptions underlying current and proposed regulatory practices in the area of agricultural biotechnology. Chapter 7 is an in-depth analysis and critique of one of the most controversial concepts invoked in both national and international regulatory contexts — that of "substantial equivalence". The Panel finds the use of "substantial equivalence" as a decision threshold tool to exempt GM agricultural products from rigorous scientific assessment to be scientifically unjustifiable and inconsistent with precautionary regulation of the technology. The Panel recommends a four-stage diagnostic assessment of transgenic crops and foods that would replace current regulatory reliance upon "substantial equivalence" as a decision threshold.

Chapter 8 focuses upon the current debate over the validity and relevance of the so-called "precautionary principle" in the regulation of agricultural biotechnologies. Many national and international regulatory bodies (including Canada) have adopted the "precautionary principle" as a regulatory axiom. In this chapter, the Panel lays out an understanding of the principle it considers to have both scientific and regulatory validity, and recommends its use as an axiom of Canadian regulatory policy. The Panel finds the use of "substantial equivalence" as a standard of safety (as opposed to a decision threshold in assessment of risk) to be, in general, a precautionary standard.

Chapter 9 raises a series of issues the Panel identified during its deliberations that it considered to be of critical importance in maintaining the integrity of science upon which the regulation of agricultural biotechnology should be based, and in maintaining public confidence in the regulatory processes. Part 1 of the chapter raises serious concerns about the undermining of the scientific basis for risk regulation in Canada due to the following factors:

- the conflict of interest created by giving to regulatory agencies the mandates both to promote the development of agricultural biotechnologies and to regulate it;
- the barriers of confidentiality that compromise the transparency and openness to scientific peer review of the science upon which regulatory decisions are based; and
- the extensive and growing conflicts of interest within the scientific community due to entrepreneurial interests in resulting technologies and the increasing domination of the research agenda by private corporate interest.

In Part 2 of Chapter 9, the Panel considers the scientific basis for mandatory labelling of genetically engineered food products, and establishes guidelines for mandatory and voluntary labelling on the basis of health risks. The Panel recognizes that there are broader social, political and ethical considerations in the debate about mandatory labelling of GM foods that lie outside the Panel's specific mandate, so this discussion is not intended to provide a comprehensive answer to the issue of mandatory labelling.

SUMMARY OF THE EXPERT PANEL'S RECOMMENDATIONS

In light of its investigations, the Panel made the following recommendations. The rationale and complete text of these recommendations are found at the end of each major section of the Report.

Recommendations Concerning Underlying Policies and Principles Guiding the Regulation of Agricultural Biotechnology

7.1 The Panel recommends that approval of new transgenic organisms for environmental release, and for use as food or feed, should be based on rigorous scientific assessment of their potential for causing harm to the environment or to human health. Such testing should replace the current regulatory reliance on "substantial equivalence" as a decision threshold.

7.2 The Panel recommends that the design and execution of all testing regimes of new transgenic organisms should be conducted in open consultation with the expert scientific community.

7.3 The Panel recommends that analysis of the outcomes of all tests on new transgenic organisms should be monitored by an appropriately configured panel of "arms-length" experts from all sectors, who report their decisions and rationale in a public forum.

8.1 The Panel recommends the precautionary regulatory assumption that, in general, new technologies should not be presumed safe unless there is a reliable scientific basis for considering them safe. The Panel rejects the use of "substantial equivalence" as a decision threshold to exempt new GM products from rigorous safety assessments on the basis of superficial similarities because such a regulatory procedure is not a precautionary assignment of the burden of proof.

8.2 The Panel recommends that the primary burden of proof be upon those who would deploy food biotechnology products to carry out the full range of tests necessary to demonstrate reliably that they do not pose unacceptable risks.

8.3 The Panel recommends that, where there are scientifically reasonable theoretical or empirical grounds establishing a *prima facie* case for the possibility of serious harms to human health, animal health or the environment, the fact that the best available test data are unable to establish with high confidence the existence or level of the risk should not be taken as a reason for withholding regulatory restraint on the product.

8.4 As a precautionary measure, the Panel recommends that the prospect of serious risks to human health, of extensive, irremediable disruptions to the natural ecosystems, or of serious diminution of biodiversity, demand that the best scientific methods be employed to reduce the uncertainties with respect to these risks. Approval of products with these potentially serious risks should await the reduction of scientific uncertainty to minimum levels.

8.5 The Panel recommends a precautionary use of "conservative" safety standards with respect to certain kinds of risks (e.g. potentially catastrophic). When "substantial equivalence" is invoked as an unambiguous safety standard (and not as a decision threshold for risk assessment), it stipulates a reasonably conservative standard of safety consistent with a precautionary approach to the regulation of risks associated with GM foods.

9.1 The Panel recommends that Canadian regulatory agencies and officials exercise great care to maintain an objective and neutral stance with respect to the public debate about the risks and benefits of biotechnology in their public statements and interpretations of the regulatory process.

9.2 The Panel recommends that the Canadian regulatory agencies seek ways to increase the public transparency of the scientific data and the scientific rationales upon which their regulatory decisions are based.

9.3 The Panel recommends that the Canadian regulatory agencies implement a system of regular peer review of the risk assessments upon which the approvals of genetically engineered products are based. This peer review should be conducted by an external (non-governmental) and independent panel of experts. The data and the rationales upon which the risk assessment and the regulatory decision are based should be available to public review.

9.4 The Panel recommends that the Canadian Biotechnology Advisory Commission (CBAC) undertake a review of the problems related to the increasing domination of the public research agenda by private, commercial interests, and make recommendations for public policies that promote and protect fully independent research on the health and environmental risks of agricultural biotechnology.

Recommendations Concerning Regulations and Guidelines

4.1 The Panel recommends that federal regulatory officials in Canada establish clear criteria regarding when and what types of toxicological studies are required to support the safety of novel constituents derived from transgenic plants.

4.3 The Panel recommends that, in view of the availability of suitable alternative markers, antibiotic resistance markers should not be used in transgenic plants intended for human consumption.

4.8 The Panel recommends that approvals should not be given for GM products with human food counterparts that carry restrictions on their use for non-food purposes (e.g. crops approved for animal feed but not for human food). Unless there are reliable ways to guarantee the segregation and recall if necessary of these products, they should be approved only if acceptable for human consumption.

5.1 The Panel recommends that the Canadian Food Inspection Agency (CFIA) develop detailed guidelines describing the approval process for transgenic animals intended for (a) food production or (b) other non-food uses, including appropriate scientific criteria for assessment of behavioural or physiological changes in animals resulting from genetic modification.

6.10 The Panel recommends that companies applying for permission to release a GM organism into the environment should be required to provide experimental data (using ecologically meaningful experimental protocols) on all aspects of potential environmental impact.

6.11 The Panel recommends that an independent committee should evaluate both the experimental protocols and the data sets obtained before approvals of new plants with novel traits are granted.

6.12 The Panel recommends that standard guidelines should be drawn up for the long-term monitoring of development of insect resistance when GM organisms containing "insecticidal" products are used, with particular attention to pest species known to migrate over significant distances.

6.13 The Panel recommends that a moratorium be placed on the rearing of GM fish in aquatic netpens.

6.14 The Panel recommends that approval for commercial production of transgenic fish be conditional on the rearing of fish in land-based facilities only.

Recommendations Concerning the Regulatory Process

4.2 The Panel recommends that regulatory authorities establish a scientific rationale that will allow the safety evaluation of whole foods derived from transgenic plants. In view of the international interest in this area, the Panel further recommends that Canadian regulatory officials collaborate with colleagues internationally to establish such a rationale and/or to sponsor the research necessary to support its development.

4.6 The Panel recommends development of mechanisms for after-market surveillance of GM foods incorporating any novel protein.

4.7 The Panel recommends that the appropriate government regulatory agencies have in place a specific, scientifically sound and comprehensive approach for ensuring that adequate allergenicity assessment will be performed on GM foods.

4.9 The Panel recommends that all assessments of GM foods, which compare the test material with an appropriate control, should meet the standards necessary for publication in a peer-reviewed journal, and all information relative to the assessment should be available for public scrutiny. The data should include the full nutrient composition (Health Canada, 1994), an analysis of any anti-nutrient and, where applicable, a protein evaluation such as that approved by the United Nations Food and Agriculture Organization (FAO).

4.10 The Panel recommends that protocols should be developed for the testing of future genetically engineered foods in experimental diets.

4.11 The Panel recommends that the Canadian Nutrient File should be updated to include the composition of genetically engineered foods and be readily available to the public.

5.2 The Panel recommends that the approval process for transgenic animals include a rigorous assessment of potential impacts on three main areas:

1) the impact of the genetic modifications on animal health and welfare;
2) an environmental assessment that incorporates impacts on genetic diversity and sustainability; and
3) the human health implications of producing disease-resistant animals or those with altered metabolism (e.g. immune function).

5.3 The Panel recommends that the tracking of transgenic animals be done in a manner similar to that already in place for pedigree animals, and that their registration be compulsory.

5.4 The Panel recommends that transgenic animals and products from those animals that have been produced for non-food purposes (e.g. the production of pharmaceuticals) not be allowed to enter the food chain unless it has been demonstrated scientifically that they are safe for human consumption.

5.6 The Panel recommends that the use of biotechnology to select superior animals be balanced with appropriate programs to maintain genetic diversity, which could be threatened as a result of intensive selection pressure.

5.8 The Panel recommends that changes in susceptibility of genetically engineered plants to toxin-producing microbes, and the potential transfer of these to the animal and the food supply, be evaluated as part of the approval process.

5.9 The Panel recommends that a data bank listing nutrient profiles of all GM plants that potentially can be used as animal feeds be established and maintained by the federal government.

5.10 The Panel recommends that university laboratories be involved in the validation of the safety and efficacy of GM plants and animals.

5.11 The Panel recommends that Environment Canada and the Canadian Food Inspection Agency establish an assessment process and monitoring system to ensure safe introductions of GM organisms into Canada, according to the intent of the Canadian Environmental Protection Act.

6.1 The Panel recommends that all ecological information on the fate and effects of transgenic biotechnology products on ecosystems required under existing regulations should be generated and made available for peer review.

6.2 The Panel recommends the carrying out of exhaustive, long-term testing for ecological effects of biotechnology products that pose environmental risks, especially with respect to

persistence of the organism or a product of the organism, persistent effects on biogeochemical cycles, or harmful effects resulting from horizontal gene transfer and selection.

6.3 The Panel recommends that, in evaluating environmental risks, scientific emphasis should be placed on the potential effects of selection operating on an introduced organism or on genes transferred to natural recipients from that organism.

6.5 The Panel recommends that the history of domestication, and particularly the time period and intensity of artificial selection, of GM plants should be taken into account when assessing potential environmental impacts. Species with a short history of domestication should receive particularly close scrutiny because they are more likely to pose environmental risks.

6.6 The Panel recommends that environmental assessments of GM plants should pay particular attention to reproductive biology, including consideration of mating systems, pollen flow distances, fecundity, seed dispersal and dormancy mechanisms. Information on these life-history traits should be obtained from specific experiments on the particular GM cultivar to be assessed, not solely from literature reports for the species in general.

6.7 The Panel recommends that environmental assessments of GM plants should not be restricted to their impacts on agroecosystems but should include an explicit consideration of their potential impacts on natural and disturbed ecosystems in the areas in which they are to be grown.

6.8 The Panel recommends that research data from experiments conducted by industry on the potential environmental impacts of GM plants used in Canadian Environmental Protection Agency assessments should be made available for public scrutiny.

6.16 The Panel recommends that potential risks to the environment posed by transgenic fish be assessed not just case-by-case, but also on a population-by-population basis.

Recommendations Concerning Scientific Capacity for the Regulation of Food Biotechnology

4.4 The Panel recommends that the Canadian government support research initiatives to increase the reliability, accuracy and sensitivity of current methodology to assess allergenicity of a food protein, as well as efforts to develop new technologies to assist in these assessments.

4.5 The Panel recommends the strengthening and development of infrastructures to facilitate evaluation of the allergenicity of GM proteins. This could include development of a central bank of serum from properly screened individuals allergic to proteins which might be used for genetic engineering, a pool of standardized food allergens and the novel GM food proteins or the GM food extracts, maintenance and updating of allergen sequence databases, and a registry of food-allergic volunteers.

5.5 The Panel recommends that federal and provincial governments ensure adequate public investment in university-based genomic research and education so that Canada has the capacity for independent evaluation and development of transgenic technologies.

5.7 The Panel recommends that a national research program be established to monitor the long-term effects of GM organisms on the environment, human health, and animal health and welfare.

6.4 The Panel recommends that a detailed analysis be undertaken of the expertise needed in Canada to evaluate environmental effects of new biotechnology products and, if the appropriate expertise is found to be lacking, resources be allocated to improving this situation.

6.9 The Panel recommends that a federally funded multidisciplinary research initiative be undertaken on the environmental impacts of GM plants. Funds should be made available to scientists from all sectors (industry, government and university) with grant proposals subject to rigorous peer review.

6.15 The Panel recommends the establishment of comprehensive research programs devoted to the study of interactions between wild and cultured fish. Reliable assessment of the potential environmental risks posed by transgenic fish can be undertaken only after extensive research in this area.

6.17 The Panel recommends that identification of pleiotropic, or secondary, effects on the phenotype resulting from the insertion of single gene constructs into GM organisms be a research priority.

7.4 The Panel recommends that Canada develop and maintain comprehensive public baseline data resources that address the biology of both its major agroecosystems and adjacent biosystems.

7.5 The Panel recommends that Canada develop state-of-the-art genomics resources for each of its major crops, farm animals and aquacultured fish, and use these to implement effective methodologies for supporting regulatory decision making.

1. INTRODUCTION

> "The risks in biotechnology are undeniable, and they stem from the unknowable in science and commerce. It is prudent to recognize and address those risks, not compound them by overly optimistic or foolhardy behaviour."
> Editors - *Nature Biotechnology* (October 2000)

THE EXPERT PANEL

This Report is submitted in response to a joint request to the Royal Society of Canada from three agencies of the Government of Canada (Health Canada, Canadian Food Inspection Agency, and Environment Canada) that an independent Expert Panel be convened by the Society to advise on a series of questions related to the safety of new food products being developed through the use of new genetic engineering technologies. The specific questions were laid out in provisional Terms of Reference provided to the Royal Society of Canada Committee on Expert Panels in January 2000. The Committee on Expert Panels then selected a group of 15 people from across Canada who represented a wide range of scientific and policy-related expertise relevant to the questions submitted. The Terms of Reference were then reviewed and interpreted at a meeting of the Expert Panel with representatives of the sponsoring government departments in March 2000. The Royal Society agreed to submit the Report of the Expert Panel to the Government of Canada by December 15, 2000. By mutual agreement this deadline was extended to January 31, 2001.

MANDATE AND TERMS OF REFERENCE

The mandate of the Expert Panel on the Future of Food Biotechnology is to provide Health Canada, the Canadian Food Inspection Agency, and Environment Canada with advice on our regulatory system and the scientific capacity that the federal government requires into the 21st century to ensure the safety of new food products being developed through biotechnology.

Terms of Reference

- To forecast:
  - the types of food products being developed through biotechnology that could be submitted for regulatory safety reviews by Health Canada and/or the Canadian Food Inspection Agency over the next 10 years;
  - the science likely to be used to develop these products; and

- any potential short- or long-term risks to human health, animal health and the environment due to the development, production or use of foods derived from biotechnology.

To assess approaches and methodologies developed in Canada and internationally to evaluate the safety of foods being developed through biotechnology, including those being developed by the World Health Organization, the Food and Agricultural Organization and the Codex Alimentarius Commission.

To identify:

- the scientific capacity that will be needed to ensure the safety of new foods derived from biotechnology, including human resources for research, laboratory testing, safety evaluation, and monitoring and enforcement; and
- any new policies, guidelines and regulations related to science that may be required for protecting human health, animal health and environmental health.

INTERPRETATION OF THE TERMS OF REFERENCE

At its first meeting in March 2000, the Expert Panel met with representatives of the sponsoring government departments to discuss and clarify the Terms of Reference. The Terms of Reference were not revised as a result of this meeting, but the discussions helped to clarify the expectations of the sponsors relative to the scope and limits of this study. The discussion with the sponsors made it evident that, although the focus of the Expert Panel's enquiry was on the scientific aspects of the new technologies and their effective regulation, the Panel would need to address many peripheral issues that touch on the question of the appropriate use of science in the regulation of risks. For example, controversies over such questions as the advisability of labelling genetically engineered food products, the impacts of international trade agreements and international standards upon Canadian food safety, the complex relationship among biotechnology industries, scientists and regulators, are all related to the question of how science should be used to manage effectively the risks associated with genetically engineered products.

The issues raised in the public debate about biotechnology range across a wide spectrum of concerns. They include concerns about impacts upon human and animal health resulting from undetected toxins or allergenic substances in GM food products, about the environmental impacts of transgenic genes proliferating in wild species of plants, about loss of biodiversity, the impact of expanded reliance upon GM crops upon the agricultural economies of less developed nations, and about impacts upon consumers resulting from monopolization in agribusiness. They also include explicitly ethical concerns about splicing genes across plant and animal "kingdom" barriers, producing "unnatural" animal chimeras, about "playing God" with nature, about the rights of

consumers to choose whether to expose themselves to unknown or uncertain risks, or just simply to choose what technologies they will support with their purchasing dollars.

These are among the concerns shaping public attitudes about food biotechnology. They are often expressed as concerns about the "risks" and "safety" of genetically engineered foods, because they are perceived as posing "risks" to a wide variety of social and personal values. The Terms of Reference ask the Expert Panel to focus its attention upon a fairly narrow portion of this wide range of issues — those related to human and animal health and environmental impacts. We have tried to respect the limits of the Terms of Reference as much as possible. Accordingly, it is important at the outset to clarify which questions we have taken as most central to our task under the Terms of Reference, and which we have left outside our consideration. However, it is important to understand that answers to questions not specifically within our mandate are often relevant to, and influence answers to questions that are within it. The health and environmental safety issues posed to the Panel in the Terms of Reference, though largely scientific in nature, often cannot be addressed fully without reference to broader ethical, political and social issues and assumptions.

The different types of concerns at issue in the public debate about biotechnology can be classified helpfully in three categories. These categories distinguish three different kinds of values feared to be placed "at risk" by various biotechnologies. These are concerns about: 1) the potential risks to the health of human beings, animals and the natural environment, 2) the potential risks to social, political and economic relationships and values, and 3) the potential risks to fundamental philosophical, religious or "metaphysical" values held by different individuals and groups. Accordingly, we shall refer to these categories of concern about biotechnology as: 1) Health and Environmental Risks, 2) Socio-Economic Risks, and 3) Philosophical/Metaphysical Risks.

We recognize that the borders between these types of questions are not always clearly demarcated, nor are the questions completely independent. Assumptions about social, economic and philosophical questions often enter into deliberations, and thus conclusions, about the magnitude and acceptability of risks. For example, a strong conviction about the extensive benefits of the widespread adoption of biotechnology crops (or the adverse consequences of failing to adopt them) will tend to colour attitudes toward safety issues (i.e. higher risk levels will be viewed as acceptable). We shall attempt in this Report to be attentive both to the distinctions between the types of issues involved in the food biotechnology debate and to those places where they interpenetrate each other.

Health and Environmental Risks

As noted in the Introduction, the Terms of Reference given to the Expert Panel for this Report ask the panel to focus its attention on this first category of concerns — those having to do with potential risks to the health of human beings, animals and the environment posed by current and projected agricultural products of biotechnology. These Terms of Reference specify that the Panel give its attention to three aspects of this question: 1) the forecasting of the food biotechnologies likely to be submitted for regulatory safety reviews, the science behind them, and the risks to human, animal health and the environment posed by these technologies; 2) the assessment of the methods developed nationally and internationally for assuring the safety of these food biotechnologies; and 3) the identification of the scientific capacities and regulatory policies relating to this science that may be required for protecting human, animal and environmental health.

The subsequent chapters of this Report will attempt to address these issues by laying out the characteristics of current and projected food biotechnologies, identifying the significant hazards potentially associated with the products of these technologies, and assessing the potential magnitudes of the risk these might pose to human, animal or environmental health. They will also evaluate and recommend the methodologies and procedures required in order for industry and government regulators to evaluate reliably the risks posed by specific biotech food products in each of these areas, and to manage these risks within safe (acceptable) levels.

Since these Terms of Reference direct the Panel's attention narrowly to one category of the issues that have emerged in the public debate about food biotechnology, it therefore goes without saying that the Panel does not intend that this Report represents a comprehensive response to the whole range of concerns involved in the question of whether the development of any particular agricultural biotechnology, or biotechnology in general, is advisable. Such a comprehensive response would have to address all three categories of concern identified above. We do offer an account of the social and economic factors driving this development but, in order to adhere to the terms of our mandate, this account is solely for the purposes of predicting the course of this development and assessing the potential problems it poses for managing health and environmental risks. Concerns of human, animal and environmental health are among the most critical ones raised in the food biotechnology debate, but they are only a small part of the debate. There are also the other categories of important questions having to do with the economic costs and benefits of agricultural biotechnology, the social impacts on societies at different stages of technological and social development, environmental and social ethics, as well as deeply held philosophical and religious convictions about human interventions in nature. While this Report comments on those issues where they are relevant to health and environmental impacts, it does not presume to address them comprehensively.

Socio-Economic Risks

The second category of concerns expressed in the public debate about food biotechnology relate to the potential risks it poses to a variety of socio-economic values: these include concerns about concentration of the seed industry in the hands of a few multinational companies, with potential dislocation of rural farm communities in favour of a few large agribusinesses. They include concern about the potential effects of biotechnology on farmers in lesser developed countries, who may be at risk of increased dependency on multinational corporations from the developed world, leading to decreasing food self-sufficiency in these areas. The recent furor over the so-called “Terminator” seed technology being developed by agbiotech companies and the USDA, which culminated in Monsanto’s announcement that the technology would not be brought into the marketplace, was generated by just this concern.

Proponents of food biotechnology argue that the socio-economic arguments in fact make the strongest case for its development. They argue that the ability to engineer food crops for greater productivity, adaptation to growth in marginal soils and climates, and enhanced nutritional qualities is essential to meeting the food needs for an expanding world population. Proponents also believe that the technology will improve food quality and lower prices for consumers everywhere. These arguments are elaborated elsewhere in this Report as part of the Panel’s discussion of the social forces shaping biotechnology development. The Panel did not, however, consider it within its mandate to assess the extent to which these claims are reliable, or to evaluate quantitatively or qualitatively the magnitude of the benefits of food biotechnology.

Because we have not made these evaluations of the claimed benefits of agricultural biotechnologies, this Report cannot be read as providing any answers to the question of whether these technologies are socially desirable in the broadest sense. Many experts argue that the “safety” (acceptability of the risks) of these technologies depends upon whether the risks, whatever they may be, are outweighed by the overriding benefits they achieve. This “risk-cost-benefit” approach to safety is only one among many safety standards that can be invoked by risk regulators. It tends to function as a less restrictive standard of safety, insofar as it permits, in principle, any level of risk as long as there are off-setting benefits. There are many other types of standards commonly advocated as well, including various “threshold” standards, procedural standards, and even “zero-risk”, which are usually more restrictive, or conservative.

This Report does not include a full discussion of this very important risk management issue. It does, however, address the concept of “substantial equivalence”, which is increasingly invoked as a safety standard as well as a risk assessment decision threshold (Chapters 7 and 8). When invoked as a safety standard, “substantial equivalence” establishes a “threshold” of acceptable risk, requiring that the risks of a GM product not exceed those of its non-GM counterpart, regardless of the magnitude of the benefits it may provide. Used in this way, the

Panel notes (Chapter 8) that "substantial equivalence" functions as a fairly precautionary safety standard.

It is evident, then, that major factors influencing the social acceptability of food biotechnology are those having to do with perceptions of the socio-economic and political impacts of the growth of the technology rather than only the questions of risk to health and the environment. These involve strongly held political and ethical values — those related to a sense of social justice in the distribution of costs, risks and benefits, individual and community rights to choose, and democratic ideals of participation in decisions concerning the development of biotechnology. While these considerations are significant factors in the overall social question of the merits of food biotechnology, since they are not centrally within the mandate of this Panel, we comment on them only where necessary to address fully the health and environmental questions within our mandate.

Philosophical/Metaphysical Risks

The public debate about food biotechnology has also included a third category of issues. These relate to concerns that genetic engineering technologies give human beings powers over nature that are deeply unethical, either in themselves or in certain of their applications. These concerns are rooted in fundamental philosophical and theological perspectives on human and animal nature, the natural environment and divinity. The concerns about food biotechnology are part of a deeper view of biotechnology in general, which is considered to involve interventions in the natural world that undermine appropriate human relationships to nature or God. It is primarily the *process* of genetic engineering that is at issue, rather than its *impacts* upon health, environment or society. It is the fact that genes are altered and transposed between organisms by processes that would not occur "naturally", crossing species and kingdom barriers and producing life forms (transgenic plants and animals) that would not by produced by the "natural" processes of evolution.

The critical operative concept here, clearly, is that of what is "natural". This concept is not a scientific one, but a normative one — a view of how human, animal and plant natures *should* be, or "how God intends them to be". The fact that a member of the British royal family can, with the support of a large number of British and European citizens, question whether human beings have the "right to play God" with GM organisms, indicates how widespread and deep-seated these metaphysical concerns can be.

These kinds of concerns about biotechnology are often expressed in less metaphysical and abstract language. They are often expressed as considerations of precaution in the face of uncertainty. Many critics of biotechnology base their arguments on the claim that current biotechnologies are based on a reductionist view of nature that is neither scientifically nor

philosophically defensible. They challenge the view that the relationships between genes and the traits of organisms are deterministic and one-to-one, arguing instead that these relationships are complex and often unpredictable, since genes act in consort with other genes, the whole organism and the environment (Heinberg, 1999). The underlying force of these claims is that, because genetic technologies are full of high uncertainties, it is morally irresponsible for human beings to "muck around" with nature in this way.

There is also a set of philosophical and metaphysical concerns that are not so much about biotechnology per se as they are about certain *implementations* of it. Many people object, in principle, to such interventions as the cloning of human beings and/or animals, the engineering of cross-species chimeras (cat-rabbits, pigs used to grow human organs for xeno-transplantation, etc.). They would not argue that all uses of biotechnology are "unnatural", but would view these kinds of uses as crossing fundamental lines of moral acceptability. Such practices may be viewed as undermining human conceptions of dignity and equality (e.g. in human cloning) or respect for nature as sacred (e.g. chimeras). In effect, these practices pose risks to fundamental moral values — or moral risks.

An even more concrete and immediate concern of this type relates to the transfer of genes from "taboo" foods into other food products. Religious and ethnic groups that observe religious dietary rules prohibiting the eating of certain animals have obvious problems with the consumption of vegetable or other animal foods that may carry genes taken from the prohibited animal. Vegetarians have similar problems with plants engineered with animal genes. These are all concerns rooted in fundamental philosophical/metaphysical beliefs about the world. This does not make them any less significant in the public debate about food biotechnology.

Again, these philosophical and metaphysical issues, while critically important in the public debate about food biotechnology and in the overall assessment of its social merits, are not taken to be within the mandate of the Expert Panel, and this Report takes no stance with respect to them, except where they may impinge directly upon the matters of health risk assessment and management that do fall within its mandate. One place where they do impinge upon these matters, for example, is in the conception of what constitutes full human or animal health. Traditionally, conceptions of health and "well-being" invoke metaphysical notions of what is "natural", "normal" or "good". For this reason, the Panel's discussion of animal health concerns in Chapter 5 requires discussion of animal welfare.

Scientific and Extra-Scientific Issues in Risk Analysis

We have categorized the different elements of the biotechnology debate as reflecting concerns about different types of values or concerns "at risk" in order to clarify certain issues that are typically confused in the debate, and to clarify our inclusion in this Report of certain matters

we consider relevant to the management of health risks. The confusion is engendered by a common, but quite different, way of distinguishing the issues. It is commonly stated that the biotechnology debate falls into the following three kinds of disagreement: 1) *scientific* disagreements about types and degrees of risk to human, animal and environmental health; 2) *political* disagreements about the social and economic impacts of agricultural biotechnology (disagreements based upon different political views); and 3) *religious, ethical and philosophical* disagreements about whether biotechnology is "unnatural", "immoral", "playing God", etc.

Our characterization of the various aspects of the debate may seem at first sight to be no different from the first. But there is a critically important difference. The common classification assumes that the various issues in the debate can be distinguished according to their *method of inquiry*. It assumes that the issues in the first category involve empirical questions resolvable primarily by means of scientific method. The issues in the second category are about preferred political and social structures, which involve matters, not only of social science and economics, but also of political and social philosophy not resolvable through scientific investigation. The issues in the third category are characterized as deeply religious, moral and metaphysical. They are not matters of physical or social science at all, but value judgments deeply rooted in culture, ethnicity and tradition, which are generally considered to be unresolvable by any rational method.

The Panel does not accept the common classification of the issues in the biotechnology debate because of its implication that the questions put to it in the Terms of Reference — having to do with the identification of potential risks to human, animal and environmental health — are purely questions of science. There is no doubt that questions about the potential hazards inherent in the products of agricultural biotechnology and the mechanisms for assessing the magnitude of the health risks they pose are primarily scientific, requiring the very best scientific methods and expertise for their resolution. But they are not purely scientific. It is now generally recognized in the scholarly literature on the nature of risk analysis that many aspects of the task of assessing the magnitude of technological risks and managing them within the limits of safety involve judgments and decisions that are not themselves strictly scientific (Salter et al., 1988; Mayo et al., 1991; Shrader-Frechette, 1991). They involve value judgments related to such issues as the appropriate way to handle uncertainties in scientific data and results, assignment of the burden of proof among stakeholders in risk issues, standards of proof, definition of the scope of the risk issue (e.g. should human error be considered part of the risk of the technology?), and, of course, the central issue, already noted, of what levels of risk should be considered "acceptable". Such "extra-scientific" judgments are inherent in any assessment of risk and in the judgments about the technological and social mechanisms for maintaining it within safe limits. Similar judgments are involved in any attempt to predict future scientific and technological developments, which are always at least partially dependent upon human choices and other undetermined variables.

The Panel recognizes that answers to the questions put to it in the Terms of Reference require the very best in scientific investigation. It is for this reason that the Panel was appropriately constituted to represent expertise from the scientific disciplines most relevant to food biotechnology — including biology, biochemistry, genetics, environmental science, ecology, medical science, animal science, food science, plant science, nutrition, toxicology, entomology, etc. But the Panel also recognizes that the Terms of Reference require investigation into the extra-scientific issues that establish the framework for the scientific investigations involved in risk analysis. For this reason, it is appropriate that the Panel membership also included specialists in the "normative" disciplines of law, philosophy and ethics.

PANEL PROCEDURES

The members of the Expert Panel were appointed by the Royal Society in February 2000. On February 17, 2000, the Royal Society of Canada issued a press release announcing the establishment of the Expert Panel, the appointment of the Panel members, and an outline of the Terms of Reference. The press release invited written submissions from any interested parties in Canada on issues relevant to its mandate and objectives. The deadline date for these submissions was set at April 30, 2000. However, in view of the fact that many parties did not receive information about the Panel process in time, this date was subsequently extended by the Expert Panel (in announcements on the Royal Society of Canada web site and in press reports) to July 31.

The Expert Panel convened its first meeting in Ottawa on March 15 and 16, 2000. During these two days, the Panel met with representatives of the sponsoring government departments to discuss and clarify the Terms of Reference. During this meeting, it became evident that two of the original appointees to the Expert Panel (François Pothier and James Germida) would not be able to fulfill all the obligations of membership on the panel. They were subsequently replaced by two alternate members (John Kennelly and Jeremy McNeil). At this meeting, the Expert Panel identified the major scientific and other issues that the Report would need to address in order to answer the questions put to it in the Terms of Reference, and a draft structure for the Report was adopted. Research assignments were parsed out to the members of the Panel for reporting at the subsequent meeting.

The Expert Panel convened a second meeting for three days, June 27 to 29, in Ottawa to consider the preliminary research carried out by the members and distributed to them prior to the meeting. In preparation for this meeting, all submissions from interested parties received by that date were read by all members of the Panel, and issues raised in these submissions were discussed as part of an extended discussion in which the members developed an inventory of the major issues to address and moved toward agreement on the position we wished to take with respect to

them, given the research findings to that date. Members left this meeting with a revised Report Outline, and research and drafting assignments for a preliminary Report to be considered at the subsequent meeting.

A third meeting was held, again in Ottawa, on August 8 to 10. At this meeting, the Panel reviewed the additional submissions from the public that had been received in response to the extended deadline of July 31. The results of the initial research and the first round of drafts assigned at the June meeting had been circulated to all members of the Panel and were carefully reviewed by the whole Panel. Additional research needs were identified as well as the preliminary overall direction of the findings.

The initial round of research had also identified a series of additional questions the Panel felt could be answered only by further consultation with personnel from the sponsoring agencies. In response to last-minute requests to the agencies, all arranged quickly to provide spokespersons to meet with us. In separate meetings, the Panel interviewed William Yan, Acting Head, Office of Food Biotechnology, Health Canada; Bart Bilmer, Director, Office of Biotechnology, Canadian Food Inspection Agency; Phil McDonald, Biotechnologist, Plant Biotechnology, Canadian Food Inspection Agency, and James Lauter, Evaluation Specialist, Biotechnology Section, Environment Canada. The Panel would like to express gratitude to these persons for agreeing to meet with us on very short notice, and for their forthcoming responses to our questions.

The final meeting of the Expert Panel took place in Vancouver on October 27 to 29. In the interim, the Panel members had been assigned additional research projects and were asked to bring revised drafts of assigned sections for critique and evaluation by the whole Panel. The Panel members reached agreement on the final revisions necessary for the Report at this meeting.

The first draft of this Report was sent to an anonymous group of seven Peer Reviewers, who were selected by the Royal Society of Canada Committee on Expert Panels. The Panel received comments from three of the Peer Reviewers in time for it to incorporate their suggestions into the final version of the Report. The comments and suggestions of the Peer Reviewers were extremely helpful to the Panel and contributed significantly to the quality of the Report.

We regret that two of the persons originally appointed to assist the Expert Panel were unable to do so due to health reasons. Dr. Thérèse Leroux, who was appointed as a member of the Panel, was unable to attend meetings or to participate in the writing of the Report due to health problems. Dr. Michael Smith, who was appointed as a special advisor to the Panel, was also unable to serve in this capacity due to ill health. The fourth meeting of the Panel was held in Vancouver with the expectation that Dr. Smith would be able to join us. We were saddened by his death only a few weeks before this meeting.

A NOTE ON TERMINOLOGY

Terms that have become part of the common currency of debate about food biotechnology do not always have a unequivocal meaning. Sometimes, the resulting ambiguity is used as a subtle tool in favour of one side or other of the debate. For example, a frequently heard argument against those who question the health or environmental safety of biotechnology is that "There is nothing new about biotechnology — human beings have been using it for centuries in the cultivation of yeasts and cultures, and in the selective breeding and hybridization of plants and animals." The force of this claim depends, of course, upon a definitional stipulation that "biotechnology" refers broadly to any technique for shaping the genetic characteristics of organisms, as well as a further assumption that new recombinant DNA techniques are no different in character or consequence from the traditional techniques. The latter, of course, is not a question that can be decided by an *a priori* definition, but only by empirical investigation.[1]

The Expert Panel sought to avoid *a priori* linguistic solutions to substantive issues. A primary substantive issue in the food biotechnology debate, and in the mandate of this Panel, is that of *whether* the new recombinant DNA technologies pose unique issues and risks requiring special regulatory expertise and techniques. We, therefore, simply state at the outset how we were using some of the key terms in the debate, so as to lend the greatest clarity and consistency to our discussion. In doing so, we do not presume to be prescribing the proper use of these terms or to be describing their most common usage. Since one of the important questions involved in the assessment of the potential hazards of these products and techniques is that of how they differ, if at all, from traditional means of modifying the genetic character of organisms, the Panel found it necessary at points to evaluate the new technologies against the traditional ones. In order to make this project transparent, we needed to adopt clear terms that refer to the different techniques.

For the purposes of this Report, therefore, the Panel uses the terms "Genetic Engineering", "Genetic Modification" and "Biotechnology" as fully synonymous terms, referring exclusively to the direct transfer or modification of genetic material using recombinant DNA techniques. They do not refer to other traditional breeding and hybridization techniques not involving these techniques. Although "Biotechnology" and "Genetic Modification" are both sometimes used to refer to all techniques of genetic modification, as defined above, we use them in the more narrow sense, assuming that this is the primary concern of both our sponsors and the general public with respect to the regulation of food biotechnology. Accordingly, in this Report, references to "Genetically Modified Organisms (GMOs)", "Genetically Engineered (GE) Foods", or "Transgenic Plants or Foods" are always only references to the products of rDNA technologies. References to non-rDNA techniques are referred to in this Report with such terms

as “selective breeding”, “artificial selection”, “hybridization” and “traditional animal/plant breeding”.

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NOTE

1. The Convention on Biological Diversity (1992), for example, defines "Biotechnology" as "any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use."

2. THE PAST, THE PRESENT AND THE FUTURE

INTRODUCTION

Genetically modified (GM) plants have already entered the food stream in many parts of the world, and large increases in acreage for a few GM crops have been observed over the last five years. However, the current generation of genetically modified organisms (GMOs) consists mostly of plants modified for a handful of traits. With the expected availability of genomic information for many species in the next few years, the floodgates of genetic modifications could open and release on the market an unprecedented variety of genetically enhanced products. In parallel with this rapid market penetration, there is increasing concern about the use of genetic engineering for food production, particularly about possible deleterious effects on human health and about the possible impacts of the widespread deployment of GMOs in the environment.

The economic stakes of agricultural biotechnology for Canada are high. We are a net exporter of agricultural products, and 26% of Canada's biotechnology companies focus on the development of agriculture and agri-food products. It is estimated that the global market for biotechnology applications will reach $50 billion annually by 2005 (Sector Competitiveness Frameworks. Bio-Industries: Part I Overview and Prospects, Bio-Industries Branch, Industry Sector, Industry Canada, March 1997), and the strongest growth is projected for the agri-food sector.

In this chapter, we examine the historical roots of GMO technology, survey its present uses in the areas of crop plants, microbes, fish, and farm animals, and make some forecasts concerning the directions this suite of technologies is likely to follow.

THE ORIGINS OF GENETIC ENGINEERING

Various species of microbes (bacteria and fungi) have for decades been modified for increased production of proteins, amino acids and commodity chemicals. Early work in this area relied primarily on discovery of naturally occurring or mutagenesis-induced variant microbial strains. Often these variant genotypes were blocked in specific metabolic pathways, or they expressed higher levels of a key rate-limiting enzyme, with the result that their metabolic output was being channelled into the desired product. Such mutant strains provided valuable biological tools for researchers, and for the fermentation industry they also represented a key commercial asset.

As our understanding of microbial metabolism expanded, the detailed structure of the pathways of interest to the fermentation industry was slowly uncovered. Many of the biosynthetic enzymes involved were identified, and the genes encoding those enzymes were eventually

isolated. Seminal to the development of genetic engineering was the discovery in the 1970s that different DNA fragments can be assembled to form new human-made DNA molecules. In 1972, a team led by Paul Berg at Stanford University used restriction enzymes to cut two DNA molecules from two different sources. They then spliced these two foreign pieces together to form a functional hybrid DNA molecule. This new molecule is referred to as recombinant DNA. Genetically modified (or genetically engineered) organisms are made of cells which contain a recombinant DNA (rDNA) molecule.

With the development of DNA manipulation techniques, it became possible to build on the knowledge of microbial biology and to create engineered microbes artificially, through direct insertion of modified genes into a desired strain, or through replacement of an existing gene. While more predictable than screening mutagenized populations, and thus potentially a more rapid path to the desired genotype, this approach had the commercial disadvantage of being accessible to anyone who had the appropriate background knowledge and training. If a competitor could create the same engineered strain within a short time, the initial developer of a new strain would have gained comparatively little commercial advantage.

The situation changed in 1980 with the U.S. Supreme Court decision (Diamond *v* Chakrabarty), which granted a patent for a GM bacterial strain specifically engineered to break down petroleum residues. This extended the legal definition of intellectual property (IP) as it provided patent protection for the first time to living organisms. In Canada, a recent decision of the Supreme Court (President and Fellows of Harvard College v. Commissioner of Patents (2000), A-334-98, Fd. Court Appeal) also paved the way for the patenting of life forms in this country. Newly engineered microbial strains thus moved from simply being trade secrets to forming part of their "owners" IP portfolio, to be traded, sold or protected by litigation, as necessary. These patented strains now comprise a vast array of "micro-reactors" whose industrial products range from amino acids, antibiotics and insulin to enzymes and alcohols.

Our Food Production System Relies on Few Genetically Selected Species

The limited array of plant and animal species that humans rely upon for most of our food supply have been genetically selected in order to improve their performance and quality. In this case, however, the process has taken place over millennia. In addition, the process, at least in its early stages, was not systematic. Nevertheless, cultivar development is essentially analogous to microbial strain improvement that has been exploited so successfully by the fermentation industry in this century. Natural mutations (and later, induced mutations) were visually identified in the wild or cultivated populations in which they occurred, and selected on the basis of their more desirable properties (better flavour, easier harvest, larger size, etc.). Only in the last hundred years was this process placed on a scientific footing, with the discovery of the genetic basis of heritable

traits. An understanding of these underlying genetic mechanisms enabled plant and animal breeders to move and combine desirable traits in a much more systematic fashion, with the result that gains in food production attributable to genetic improvement in agriculture soared in the 20th century. As a result of these efforts, there are virtually no food products on supermarket shelves that have not been improved by plant breeders (fiddleheads and wild blueberries are examples of a few remaining unimproved food plants). Plant and animal breeding have contributed enormously to our current standard of living by ensuring a generally abundant and nutritious food supply, but the plants we consume daily are significantly different from their original wild forms.

To create new plant varieties, breeders have relied on making sexual crosses between individuals that possess desirable characteristics. They then examine the progeny from these parents, looking for individuals that combine as many of the favourable characteristics as possible from each of the parents. Several such selected individuals will typically be crossed with other genotypes, or self-fertilized, to create further progeny generations, each of which will be tested for performance and quality. Nevertheless, conventional plant breeding that relies upon pollen transfer has remained a relatively slow process, and one that depends on chance for the creation of assortments of improved allelic combinations. Each cycle of improvement in a given species usually requires carrying out large numbers of controlled crosses between promising parental types, and years of work to select and evaluate the resulting progeny.

In a few cases, the products of classical breeding methods have generated products with undesirable effects on human health. There are two examples of potato varieties that were conventionally bred, but had to be withdrawn because of unacceptably high levels of glycoalkaloids. The first is the Lenape variety, which was bred from a wide cross between *Solanum tuberosum* and *S. chacoense*; it was never released for commercial purposes (Zitnak and Johnston, 1970). The second variety had been released on the market in Sweden, but was later withdrawn (Hellenas et al., 1995). An analogous problem was detected with a celery line that was bred and almost released for commercial purposes. It was found to induce contact dermatitis in field workers and chemical analysis showed that high levels of furanocoumarins were accumulating in this genotype (Trumble et al., 1990)

DIRECT GENE TRANSFER WITHIN AND BETWEEN SPECIES

Because DNA has fundamentally the same gene-coding properties whether it comes from bacteria, salmon or plants, the same molecular biology tools that have enabled extensive gene modification in microbes have been applied to the isolation and manipulation of plant and animal genes. However, moving modified genes efficiently into plant or animal genomes is much more difficult than the corresponding manipulations of microbial genomes. Research into the nature of a common plant disease in the late 1970s led to the discovery of a naturally occurring gene transfer

system for plant systems. The bacterial pathogen that causes "crown gall" disease on many plants, *Agrobacterium tumefaciens*, is able to successfully colonize its host plant because it can transfer a small set of its own genes directly and permanently into the host plant genome (i.e. it transforms part of the plant) (reviewed by Nester et al., 1984). Once established in the plant genome, these bacterial genes take over part of the metabolism of the infected cell and redirect it in a way that provides shelter (the visible gall) and sustenance specifically for the invading bacteria.

The initial report of this natural gene transfer inspired a wave of related research, out of which came the discovery that the *Agrobacterium* gene transfer process was largely insensitive to the nature of the genes being transferred. As long as a few key portions of the transferred *Agrobacterium* DNA (T-DNA) were included, the gene transfer process was capable of inserting into the plant cell genome any other "piggybacking" DNA (Chiton et al., 1980). This could include other genes obtained from plants, animals or microbes. In effect, researchers found that it was possible to "hijack" the *Agrobacterium* system and develop it as a vehicle for transferring new genes into plants.

One limitation of the *Agrobacterium* gene transfer system is the fact that *Agrobacterium* is not equally enthusiastic about infecting all species of plants. Large groups of commercially important plants, notably the cereal grains and conifers, are not hosts for *Agrobacterium* and the gene transfer system therefore does not work well in these plants. It appears, however, that while plant transformation using *Agrobacterium* is an efficient process in some plants, the actual incorporation of foreign DNA into a plant genome does not absolutely require this bacterial gene transfer system. Simply coating the foreign DNA onto microprojectiles (e.g. tiny gold beads), and blasting these into living plant cells at high velocities, will also work (Paskowski et al., 1984). The DNA coat is presumed to leach off the microprojectile surface once it is inside the recipient cell, and a small fraction of the DNA becomes incorporated into the cell's genome through a largely unknown process. This gene transfer technique has a much lower efficiency than does *Agrobacterium*-mediated gene transfer, and the incorporated DNA sequence has often been reorganized by the time it is stably inserted into the plant genome (Kohli et al., 1998). Nevertheless, the "gene gun" method has one advantage – it will, in principle, allow any plant species to be transformed, including those that are not suitable hosts for *Agrobacterium*.

SELECTING A TRANSFORMED PLANT

Both the *Agrobacterium* and "gene gun" methods are capable only of transforming a very small percentage of all the cells in the piece of plant tissue being treated. In order to create a transformed plant made only of cells carrying the new gene, two further steps must be successfully completed. First, a plant must be regenerated that is solely derived from one or more of the original transformed cells and, second, in this process all non-transformed cells must be eliminated.

Plant regeneration (i.e. the process of generating a full-size plant from a single cell) often proves to be more difficult than the actual gene transfer process itself. While the latter technologies are now routine, our understanding of the process of plant regeneration remains largely empirical. Even different varieties of a plant species often differ drastically in their ability to be regenerated from small starting tissue pieces (see e.g. Puddephat et al., 1996), so that procedures need to be customized for each new genotype of interest.

Elimination of untransformed cells is normally accomplished by adding a second gene called a selectable marker to the transferred DNA. The selectable marker gene typically encodes an enzyme that will be expressed in every transformed cell, and will confer on that cell the ability to survive in the presence of a selection agent (a chemical capable of killing plant cells). This selection agent can be an antibiotic, herbicide or other anti-metabolite. If the selection agent is an antibiotic, for example, the selectable marker gene might encode an enzyme that is designed to destroy that type of antibiotic and thus allow the cell to avoid being poisoned. As a consequence of the metabolic protection provided by the selectable marker gene, when the treated plant tissue is placed on a growth medium containing the selection agent only those cells that have been transformed will survive, while non-transformed cells will die. This selection process is often combined with the regeneration process, so that the only regenerated plants recovered are those that arose from transformed cells. Since all the cells in the regenerated plant are ultimately derived from that one progenitor cell, and their genes (including the new transgene(s)) are duplicated and shared at each cell division, the transgene(s) will now be present in every cell of the new transgenic plant. In the first generation of commercial GMO crops, the new genes inserted into the plant have always included a selectable marker gene, most commonly either an antibiotic resistance gene or a herbicide resistance gene.

The insertion of single genes into plant genomes using either the *Agrobacterium* or gene gun procedures is now a routine laboratory procedure, and the earliest commercial products of crop genetic engineering have been derived from insertion of single transgenes into important crop species. However, it should be emphasized that the initial population of transformed plants created in the laboratory by these methods is far from homogeneous. Both of the common gene transfer techniques lead to near-random insertion events (i.e. the location of the new gene within

the recipient genome cannot be predicted) (Kohli et al., 1998). Therefore, each transformed individual will carry the transgene at a different location within its genome. In many cases, they will carry multiple copies of the transgene, some of which will be functional while others may not be.

Sorting through the transgenic population and identifying those individuals that appear to express the transgene in an appropriate and useful manner requires considerable time, effort and expertise. Eventually, a limited number of lines that display the desired trait in the laboratory or greenhouse trials in a stable manner will be chosen for more extensive testing and analysis, including field trials for a number of years at multiple locations. The latter program is very similar to the evaluation process by which new crop varieties generated through conventional breeding are assessed for their ability to perform better than existing varieties, but field trials for transgenic varieties in Canada require formal approvals from the relevant regulatory agencies (see Chapter 3).

It is important to note that the utility of this process of gene transfer (genetic engineering) is largely dependent on its integration into conventional breeding programs, where it can provide a source of genetic variation. The agronomic acceptability of a transgenic variety thus derives in large part from the quality of the parental germplasm into which the transgene has been incorporated. Since the success of any crop breeding program in creating highly selected and well-adapted breeding lines is based on having access to a wide range of genetic resources, it is a key priority for plant breeders to ensure that a high level of genetic diversity is maintained in the species of interest, and its relatives.

CURRENT PRODUCTS AND FUTURE DEVELOPMENTS

GM Plants

The three types of GMO crops that first received approval for commercial release in Canada were all designed to address field-level problems faced by growers of large-scale field crops. Herbicide-resistant crops have been promoted as tools to potentially simplify weed control over large monoculture plantings and permit growers to use herbicides less damaging for the environment. Insect damage control through use of *Bt* gene-containing crop varieties (*Bt* is derived from a natural insecticide produced by bacteria) have also been promoted as tools to allow farmers (for some crops in some regions) to reduce the number of applications of pesticides. Virus-resistant crops may decrease the need for pesticides for control of insects that transmit the virus from plant to plant. The rapid adoption of GMO crop varieties by growers in Canada can be interpreted as evidence that these first-generation products have provided positive financial and/or management outcomes for the farmer.

The very first GMO varieties approved for commercial release represent the initial output of a development and testing process that usually takes at least five years to complete. The large majority (92%; Ferber, 1999) of GM crops planted in 1999 were modified for only two characteristics: either herbicide resistance or insect resistance. While there are thus relatively few functionally proven genes available for plant genetic engineering, there are dozens of candidate products at various stages in the development "pipeline". The DNA sequence of the first plant genome to be fully characterized has recently been completed (Arabidopsis Genome Initiative, 2000), and the genome of more agriculturally relevant species are expected in the coming few years. Knowledge of these complete DNA sequences will accelerate the identification of the function of many more genes, and concurrent applications of that knowledge in crop improvement.

Canada is the third largest grower of GM crops in the world (behind the US and Argentina). Canadian food safety approvals have been granted for at least 45 plants with novel traits, including canola, corn, tomato, potato, soybean, cottonseed and squash (CFIA, 2000). The number of different plant-transgene combinations tested in field trials continues to increase: 178 submissions for field trials were made in 2000 versus 40 in 1990. However, many of these products are essentially variants on the initial introductions. Since specific crop varieties are often better adapted to different soil, climate and pest situations, they will usually perform best in specific conditions. A transgene whose addition to the genome can superimpose a useful new trait can therefore be moved relatively easily into an array of agronomically well-adapted genotypes, either by breeding or by transformation of the relevant existing varieties. In this way, genetic engineers can take advantage of the classical breeding efforts that created the well-adapted lines in the first place. A substantial part of the second wave of GMO products thus consists of a wider range of crop varieties carrying herbicide resistance, Bt or anti-viral transgenes, or a combination of these.

However, other GMO traits are also beginning to reach the commercial release stage. Some of these are intended to directly address grower concerns, such as transgenes that confer resistance to fungal or bacterial disease, increased nematode resistance, enhanced frost tolerance or increased photosynthetic efficiency (CFIA, 1999). Other transgenes may modify plant fertility in specific ways that greatly simplify the production of hybrid seed, thus allowing farmers to benefit from the productivity gains associated with hybrid vigour (CFIA, 1999). Future developments will likely include varieties improved for altered flowering time, modified edible oil profiles, increased productivity, enhanced disease resistance, increased resistance to environmental stresses and improved product quality. Plants may be used for the production of compounds with a variety of uses, from pharmaceuticals to precursors of plastics.

Noticeably absent from the first generation of GM crops have been varieties that bring direct consumer benefits. It is thus ironic that the first plant product derived from biotechnology to be put on the supermarket shelves in the US was the Flavr-Savr tomato, marketed in the US in 1985 by the company Calgene. This tomato variety was created to satisfy consumer demand for a flavourful product year-round. By increasing firmness of the fruit, the tomato could be left to ripen on the vine and still be transported to market without the losses associated with a soft ripe tomato. Firmness was increased by genetically reducing the activity of an enzyme (polygalacturonase) involved in fruit softening. However, the Flavr-Savr variety was not a commercial success, and most agbiotech companies focused their initial efforts on the major American field crops, notably corn, cotton and soybeans.

Few transgenic plants currently contain more than two or three genes. A number of transgene combinations are in trials, where traits such as herbicide resistance and fertility management are "stacked" in one variety. However, most scientists agree that many important crop plant characteristics result from the combined action of many genes, sometimes as many as several dozens. Current gene transfer techniques tend to be limited in the size of the new DNA they can efficiently insert into the recipient. These limits are likely to be overcome in the near future, with the advent of new systems for transfer of very large DNA sequences, up to the size of partial or full chromosomes (Hamilton et al., 1996; Wordragen et al., 1997). However, it remains to be established whether rational design of such large gene combinations can create effective and predictable new biological functions in a transgenic plant.

Further back in the GMO pipeline can be found a much wider array of products, some of which are intended to directly address consumer preferences. These include food crops with controlled ripening, altered flower colour, increased protein content, reduced allergenicity, non-bruising and higher vitamin and mineral content. An example is the introduction of genes that produce beta-carotene (the precursor of vitamin A) in rice. The resulting "golden rice" potentially contains sufficient beta-carotene to meet human vitamin A requirements from rice alone (Ye et al., 2000).

There is also great commercial interest in the use of transgenic plants to produce industrial enzymes, pharmaceutical peptides, vaccines and other proteins of pharmaceutical interest ("molecular pharming"). For example, the enzyme lysozyme, which was previously isolated from excess egg whites, can now be produced at a far lower cost as a recombinant protein in transgenic corn.

Finally, the potential now exists to replace many microbially derived animal feed additives in current use with plants that have been GM to directly enhance the animal's feed supply. For example, a good supply of sulphur-containing amino acids is important for wool-producing

ruminant animals. To address this need, it may be possible to express in a transgenic forage crop a novel protein of particularly high cysteine content.

All of these developments are the result of insertion of genes that either express new proteins (and thus new enzymatic properties), or express a "silencing" version of an existing gene in the transgenic plant that is able to reduce the effect of the native gene. Other changes in transgenes under development include the use of selectable markers that are not based on antibiotic resistance genes (e.g. Kunkel et al., 1999), and the use of transgene constructions that allow the selectable marker to be either functionally silenced once it has performed its task during the gene transfer process, or entirely deleted from the transgenic plants (Zubko et al., 2000).

The first generation transgenic crops almost all use a strong viral gene promoter to ensure that high levels of gene effect are created in the plant. However, this promoter typically induces constant gene expression in all parts of the plant. More sophisticated gene control mechanisms are now being tested which allow the transgene to be expressed only in specific tissues of the GMO plant, or at specific times in the plant's life cycle. This capability will allow the transgene product to be targeted to the tissue where it is maximally effective, and suppresses gene product accumulation in other tissues or at other times. This could reduce internal and external collateral impacts (e.g. Bt toxin production is not needed in pollen and can create negative effects in the biosphere), and could also reduce the metabolic cost to the plant of having to accumulate products unnecessarily. Other gene control systems (inducible promoters) force the transgene to remain silent until the plant is subjected to particular treatments (e.g. sprayed with an inducer chemical) or growth condition (e.g. drought, frost, insect feeding) (e.g. Zuo and Chua, 2000).

Plant genetic engineering is presently used almost exclusively to place new genes into plant genomes with the intent of adding a novel genetic capability to the plant, or increasing or decreasing the activity of a pre-existing gene in the plant. The largely random nature of transgene insertions associated with current methods makes it impractical to consider directed gene replacement (i.e. specifically replacing an existing gene with a modified incoming version of that gene). However, it is clear that the technology is developing rapidly to allow targeted gene insertions, which will allow for more subtle changes in a plant's genotype. For instance, altering a single amino acid in a protein sequence can have a marked effect on the cellular function of that protein, and thus produce significant changes in metabolism or physiology. To engineer such an alteration of the resident copy of a gene requires changing only one or two bases in the DNA structure of the existing gene, a technically demanding process that will nevertheless probably become feasible within the next 10 years. Replacement of an existing gene with an introduced engineered gene (homologous recombination) has already been achieved on an experimental basis in *Arabidopsis* while the use of mutagenic oligonucleotides has been shown to create targeted

single base changes in plant DNA (Beetham et al., 1999; Zhu et al., 1999), albeit with low efficiencies in both cases.

These approaches allow genetic changes to be made on the resident copy of existing genes, as in many naturally occurring mutations. No new functional genetic elements (e.g. transgenes) are thus introduced into the genome, and since the existing gene control elements remain unaltered, no novel gene promoters have to be incorporated.

GM Microbes

As discussed earlier, genetically engineered microbial strains are already a component of current fermentation technologies. Enrichment, isolation and modification of naturally occurring microorganisms ("bioprospecting") will likely continue to be a source of biological material for production of enzymes, industrial chemicals and pharmaceuticals, as well as a source of novel genetic material. In agriculture, amino acid supplements (e.g. lysine, threonine and tryptophan) and many of the enzymes used to enhance the nutritive value of animal feeds (e.g. phytase, ß-glucanase, arabinoxylanase, proteinase, cellulase) are produced by fermentation, often with GM organisms. Live, GM bacteria and their products can also be used in feed harvest, storage and processing. For example, GM *Lactobacillus* sp. are used in silage production to control the aerobic and anaerobic phases of fermentation.

In a variation of the bioprospecting approach, methods have been developed for rapid screening of DNA randomly isolated and cloned directly from environmental samples without prior isolation of the organisms. Since only a small fraction of microbial species in natural environments appear to be culturable, this direct sampling can provide an array of genetic material for biotechnological applications.

While individual optimized microbial strains can be extremely useful, defined microbial consortia, containing two or more known species or strains of microorganisms, could offer greater potential. Such consortia are already applied under defined conditions in industrial processes such as dairy product manufacturing, but a wider suite of applications is likely in the future. Increased emphasis on recycling and reusing waste products of various industries and the residential waste stream could result in treatment processes based on the activities of defined, engineered microbial consortia. Examples may include upgrading or modification of wood wastes derived from forestry and pulp and paper manufacturing, petroleum refining byproducts, agricultural wastes and mining wastes.

Undefined microbial consortia are also used extensively, and could be engineered using gene transfer. "Community engineering" of this sort is currently done using donor strains to introduce mobile genetic elements (plasmids and/or transposons) containing specific genes or operons into microbial communities. This approach allows biotechnologists to modify community

function in a way that mimics the natural processes of gene exchange that occur when microbial communities are selected for particular functions using traditional enrichment methods.

Plant–microbe interactions have long been exploited for enhanced agricultural production. Plant growth-promoting rhizobacteria and mycorrhizae are presently used as seed or root inoculants to enhance plant growth. Forest tree mycorrhizae and mycorrhizae-bacteria associations are also gaining use. Future developments are likely to include the use of engineered microbial inoculants with improved ability to enhance growth, nutrient and water absorption, and stress tolerance. Since these undefined communities of microorganisms interact intimately with host plant tissues, the process of genetic modification could involve either the plant or the bacteria that are attracted to these root cells during plant growth, or both (O'Connell et al., 1996). For example, engineering the plant to produce specific chemoattractants and/or growth substrates in root exudates, and at the same time engineering bacteria to specifically recognize these signals and/or grow in response to them, would allow researchers to establish a defined plant–microbe interaction in the soil. Many uses of these engineered symbioses can be envisioned:

- enhancing phytoremediation of toxic organic chemicals and metals,
- controlling gene expression in rhizosphere bacteria by engineering plants to release specific effectors,
- bolstering the presence of disease-suppressive microbial populations, or
- enhancing nutrient uptake by encouraging the growth of microorganisms that mobilize and absorb nutrients such as phosphate or nitrate.

Another undefined microbial community important to food production exists in the rumen of some major farm animal species (e.g. cattle). The introduction of the tetracycline-resistant Tc^R a gene into *Prevotella ruminicola* was the first transfer of a gene into rumen bacteria (Flint et al., 1988). Since then, gene transfer has been used to introduce cellulase activity into a number of hind-gut bacteria, acid tolerance into cellulolytic rumen bacteria, improved protein (essential amino acid) yield by rumen bacteria and hydrogen scavenging to reduce methanogenesis in rumen bacteria. The current limitation to this technology appears to be ability of the GM organism to become successfully established in the natural rumen or hind-gut environment.

GM Animals

Animal cells generally lack the totipotency of plant cells (i.e. it is not possible to regenerate a fully differentiated animal from a single somatic cell). This precludes the use of low frequency transformation methods that rely upon chemical selection and regeneration. Researchers have therefore relied primarily on direct injection of new DNA into the nuclei of the host organism at a very early stage of development, a procedure that is both technically demanding and limited in its through-put. However, the recent development of methods of

somatic cell nuclear transfer, and the production of clones from these somatic cells for livestock species, indicates that the limitations of pronuclear microinjection for the production of GM farm animals may soon be overcome.

The context for technology development in animal production systems has also been different than the situation in plants. Reproduction technologies in large animals are less developed or inefficient compared to plants. Trait manipulation will require a more complete understanding of the genetic basis of animal biology than is presently available. In addition, with the exception of fish and poultry, populations of animals tend to be replaced slowly, and distribution channels of genetic material are more local and diffuse. These characteristics restrict the potential for the rapid and large-scale market penetration by GM genotypes, such as has occurred in crop systems. Industry investment is limited both by this, and by the challenge of maintaining control of any modified germplasm they may create, since there is no equivalent to Plant Breeders Rights in the animal industry. This latter issue may change, however, with advances in genetic marker technologies that will allow precise genotyping and identification, and the recent Supreme Court of Canada decision that allowed patenting of animal life forms.

Fish

Work on transgenic fish has focused on the development of enhanced phenotypes for the aquaculture industry, the study of gene regulation and function, developmental genetics, and the use of animals for production of human hormones such as insulin.

Research on transgenic fish has occurred at a very rapid pace. Beginning with the first report of a transgenic fish in 1985 (Zhu et al., 1985), 13 species had been GM for purposes related to food production and scientific study by the late 1980s (Kapuscinski and Hallerman, 1991), 17 by the mid 1990s (Sin, 1997), and as many as 35 in 2000 (Table 1; Devlin, 2000; Reichhardt, 2000). The first application to be made in North America for the commercial production of a transgenic fish (growth-enhanced Atlantic salmon, *Salmo salar*) was made in early 2000 in the United States (Niiler, 2000).

Means of introducing transgenes into fish

The method most commonly employed to introduce novel gene constructs into fish is microinjection of the transgene into the cytoplasm of the developing embryo (MacLean and Rahman, 1994). Millions of copies of the transgene are injected as soon as possible after fertilization, usually at the one- or two-cell stage. Because of the large size of their nuclei, unfertilized oocytes of the medaka (*Oryzia latipes*) have been microinjected directly into the nucleus (Matsumoto et al., 1992). Based on a broad examination of work on genetically engineered salmonids, Devlin (1997) found that retention rates of micro-injected novel DNA by

recipient fish can be as high as 50% among individuals that have recently resorbed their yolk sac, declining significantly to rates of 1% to 5% among individuals 6 to 12 months of age. Novel genes can also be introduced into fish via electroporation, a procedure in which fertilized eggs, and occasionally sperm (Sin et al., 1993), are immersed in a solution containing foreign DNA and then subjected to electric pulses (Inoue and Yamashita, 1997). The likelihood of successful transfer of foreign DNA by electroporation is typically very low, although it has been reported to be as high as 7% in surviving embryos (Inoue and Yamashita, 1997). Gene constructs can also be introduced via transfection of novel genes into embryonic stem cells, followed by their reintroduction into the inner cell mass of the developing embryo. Although this method may allow for precise manipulation of host genes, embryonic stem cell research in fish is still in its early stages (Devlin, 1997).

Table 1. Examples of fish that have been successfully genetically engineered.

Species	Reference
rainbow trout (*Oncorhynchus mykiss*)	Chourrout et al. (1986)
cutthroat trout (*O.clarki*)	Devlin (1997)
chinook salmon (*O. tshawytscha*)	Devlin (1997)
coho salmon (*O. kisutch*)	Devlin et al. (1994a)
Atlantic salmon (*Salmo salar*)	Fletcher et al. (1988)
brown trout (*S. trutta*)	Sin (1997)
Arctic char (*Salvelinus alpinus*)	Pitkanen et al. (1999)
African catfish (*Clarias gariepinus*)	Müller et al. (1992)
channel catfish (*Ictalurus punctatus*)	Dunham et al. (1987)
Indian catfish (*Heteropneustes fossilis*)	Sheela et al. (1999)
Japanese medaka (*Oryzias latipes*)	Inoue et al. (1990)
zebrafish (*Danio rerio*)	Stuart et al. (1988)
common carp (*Cyprinus carpio*)	Chen et al. (1993)
tilapia (*Oreochromis niloticus*)	Brem et al. (1988)
northern pike (*Esox lucius*)	Gross et al. (1992)
goldfish (*Carasius auratus*)	Zhu et al. (1985)
silver crucian carp (*C. auratus linda*)	MacLean et al. (1987)
red crucian carp (*C. auratus auratus*)	Sin (1997)
mud carp (*Cirrhinus chinensis*)	MacLean et al. (1987)
wuchang bream (*Megalobrama amblycephala*)	MacLean et al. (1987)
loach (*Misgurnus anguillicaudatus*)	Zhu et al. (1986)
mud loach (*M. mizolepis*)	Nam et al. (2000)
gilthead seabream (*Sparus auratus*)	Knibb (1997)
blackhead bream (*Acanthopagrus schlegli*)	Sin (1997)
largemouth bass (*Micropterus salmoides)*	Goldburg (1998)
striped bass (*Morone americanus*)	Goldburg (1998)
killifish (*Fundulus* sp.)	Khoo (1995)
walleye (*Stizostedion vitreum*)	Khoo (1995)

Development of growth hormone gene constructs for commercial food production

Initial research on transgenic fish in Canada focused on the transfer of an antifreeze protein gene from marine fish (e.g. winter flounder, *Pseudopleuronectes americanus*) to a commercially viable fish in the aquaculture industry, Atlantic salmon (*Salmo salar*) (Fletcher et al., 1988; Shears et al., 1991). Although the expansion of salmon aquaculture to the cold, coastal waters of the Northwest Atlantic remains one goal, most aquaculture-related research on transgenic fish has focused on the use of gene constructs to promote unregulated growth (Devlin, 1997). The commercial motivation for this work lies in the significantly reduced period of time required to rear fish to market size. The increase in growth rates achieved by transgenic fish (typically 200% to 600%, depending on the species) greatly exceeds the 5% to 10%, one-generation increases commonly achieved by artificial selection (Dunham and Devlin, 1999). Despite these large increases in growth rate, these transgenic fish do not attain final sizes greater than those achieved by non-transgenic fish. The transfer and expression of gene constructs to promote unregulated growth has now been reported for at least 15 species of fish (Dunham and Devlin, 1999; Pinkert and Murray, 1999). In Canada, research on growth hormone gene constructs has focused almost entirely on salmonids, notably Atlantic and Pacific salmon.

Future applications

Given the recent application for a GM, growth-enhanced Atlantic salmon in the United States, it is reasonable to expect that a similar application to CFIA for a growth-enhanced salmon will be forthcoming. There are, however, several other genes or gene products that have been, or are likely to be, the focus of research on genetically engineered fish in aquaculture. Novel gene constructs in fish that may form part of an application to CFIA during the next 10 years could include genes that:

1. cause over-expression of hormones such as prolactin that are involved in the transformation of anadromous fish from salt to fresh water, thereby making it theoretically possible to raise marine fish in fresh water;
2. change the pattern of expression of gonadotropin genes to allow for manipulation of the length of reproductive cycles;
3. expand the tolerance of aquaculture fish to wider ranges of environmental conditions;
4. modify the biochemical characteristics of the flesh to enhance nutritional and/or organoleptic qualities;
5. improve host resistance to a variety of pathogens;
6. control sexual maturation to prevent carcass deterioration near the end of the life cycle in Pacific salmon;
7. control sex differentiation and sterility; and

8. enable fish to use plants as a source of protein.

Shellfish and Aquatic Plants

Research on transgenic shellfish (e.g. mussels, abalone, clams) and aquatic plants is less developed than that on transgenic fish. The first successful gene transfer in a bivalve mollusc was the introduction of retroviral vectors into the dwarf surf clam (*Mulinia lateralis*) (Lu et al., 1996). Another species in which considerable research has been undertaken is the Japanese abalone (*Haliotis diversicolor*) into which growth hormone (Powers et al., 1997) and other gene constructs (Tsai et al., 1997) have been introduced. Gene constructs have also been introduced into Pacific oyster (Cadoret et al., 1997). In marine plants, there is a report (Kuebler et al., 1994) of transfer of a reporter gene into the protoplasts of *Porphyra miniata*, a commercially important red algae in southeast Asia.

The temporal lag in research on transgenic shellfish and marine plants will almost certainly translate into a similar lag in the time that will elapse before approval is sought from CFIA for the commercial production of GM shellfish or algae. Despite this lag, it is not improbable that a request will be made to CFIA within the next 10 years.

Farm Animals

Over the next five to ten years, much of the biotechnology research and development will be driven by corporate strategies to capture the potential economic value of transgenic technology for increased growth rate and altered carcass composition in meat-producing animals and compositional modification of milk and eggs.

A critical requirement to realize commercial application of genetic modifications, particularly for traits like fertility and disease resistance that are controlled by many genes, is the development of better genetic tools. Genomic analysis technologies have recently become integrated into research on all livestock species. Once the information (i.e. identity of genomic regions that encode quantitative trait loci of economic importance) and technologies (e.g. cell culture-based transgenesis) are in place, there is little doubt that breeding companies will be in a position to offer animals bred from proprietary germplasm. Such animals will have traits conferring production efficiency, or will in some way meet consumer demand, for example, by offering improved nutritional value.

Another potential application of transgenic technology in livestock production is to increase the safety of animal products for human consumption through strategies that might increase disease resistance. Genetic modifications could reduce product susceptibility to spoilage or bacterial contamination. The recent demonstration in mice, using a gene knockout strategy, of the inactivation of the prion gene involved in transmissible spongiform encephalopathies (TSE)

(Flechsig et al., 2000), raises the possibility that similar genetic modifications may be achieved in livestock species to reduce their susceptibility to specific diseases (e.g. to prevent scrapie in sheep).

NEED FOR A BROADER RESEARCH AGENDA

Agricultural biotechnology is an input industry where products are developed and priced to cover the costs of research and development. Many argue that conversion from industrial agriculture to more sustainable systems that depend less on chemicals for their productivity would eliminate the need for some of the currently projected products of biotechnology. There are probably alternatives to some biotechnology products; many of these alternatives are likely not other products, but instead the systems and methods of sustainable agriculture. It seems likely that much more research and discussion will be required to enable society to make informed choices between these alternative approaches to food production. This exploration will need to address both societal concerns about how food is produced, and assessment of "global" (or societal) costs of the choices to be made.

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3. THE REGULATORY ENVIRONMENT

INTRODUCTION

Immediately following the discovery of recombinant DNA techniques, concern was expressed within the scientific community about the potential of these technologies for creating unpredictable risks to humans or the environment. The initial response, arising from a gathering of scientific experts at Asilomar, California (Berg et al., 1975), was a self-imposed moratorium on extension of the technologies until the associated risks could be better assessed. During the period of this moratorium, extreme caution was employed in creating and handling recombinant microorganisms, and numerous studies examined their potential for presenting unanticipated phenotypes, and for modifying and transmitting the recombinant genomes.

As evidence accumulated that these organisms, while novel, could be managed and controlled using the same well-established procedures used for safely handling naturally occurring microorganisms, restrictions on contained uses of recombinant microbes gradually eased. Governments at various levels developed regulations designed to ensure public safety, based on extensive knowledge of the characteristics and behaviours of recombinant DNA in microbial systems. The resulting local and international regulatory environments have now operated successfully for over two decades, and have allowed commercial exploitation of the power of this technology in the fermentation industry, as described in Chapter 2. It is worth noting, however, that environmental release of GM microbes remains severely restricted.

With the more recent development of transgenic technology for plants and animals, a new set of challenges has faced government regulatory agencies. Compared to most microbes, crop plants, farm animals and fish are much more complex organisms, and they are generally produced and maintained directly in the outdoor environment, rather than in a confined laboratory or fermentor tank. They are also more recognizably part of both the human visual landscape and our food supply system. As governments have struggled to deal with these challenges, themes such as "substantial equivalence" and the "precautionary principle" have come to dominate the debate, and these are explored more fully in Chapters 7 and 8 of the Report. In this chapter, we present a brief overview of the current regulatory environment in Canada, both as a reference point for the Report and to help highlight some of the problems encountered in regulation of GM products.

CANADIAN REGULATION OF FOOD BIOTECHNOLOGY

Overview

In Canada, a GM product may undergo assessment by several agencies, but the Canadian Food Inspection Agency (CFIA) plays the lead role. CFIA has direct responsibility for any necessary field trials for crop plants, and for approval of any GM feed for animals. Health Canada, on the other hand, has responsibility for assessment of food safety. CFIA operates under the powers of the Seeds Act, the Plant Protection Act, the Feeds Act, the Fertilizer Act, and the Health of Animals Act. It also shares some responsibilities with Environment Canada under the Canadian Environmental Protection Act (CEPA), and with Health Canada under the Pest Control Products Act (PCPA) and the Food and Drugs Act. The Canadian Environmental Protection Act is umbrella legislation that is apparently intended to serve as a regulatory "safety net" for any biotechnology products not currently regulated by another federal act.

The Department of Fisheries and Oceans regulates aquatic organisms under the powers of the Fisheries Act, although it has not yet adopted specific regulations that address GM organisms. Since the issue of transgenic fish raises particular concerns, these have been explored in depth in Chapter 6, Part 4 of the Report.

Canadian Food Inspection Agency

CFIA has responsibility for regulating GM plants, assessing their impact on the environment and biodiversity, including the possibility of gene flow and impact on non-target organisms, and is responsible for ensuring livestock feed safety, including feed composition, toxicology, nutrition and dietary exposure (CFIA a). In April 1999, the Canadian Food Safety and Inspection Bill (C-80) was introduced into the House of Commons to "revise and consolidate certain Acts respecting food agricultural commodities, [and] aquatic commodities" and to amend the various acts under which CFIA operates (House of Commons, 2000).

CFIA is the agency that has the first contact with a biotechnology firm wishing to introduce a new GM crop plant. To obtain permission to proceed with confined field trials, the firm first applies to CFIA. The application documents must provide information on the identity and history of the plant, including any known toxins, and on the nature of the novel trait and the transformation method. The engineered DNA fragment (transgene) must be described, as must the pattern of expression of the transgene, any altered plant characteristics and evidence for stability of the novel trait. With respect to the proposed field trial, any related indigenous species must be identified, and a management plan presented that details methods to ensure reproductive isolation, describes spraying regimes, harvesting practices, proposed post-trial land use, contingency plans, and methods of site monitoring, as well as plans for providing public notification of the field trial (CFIA, 2000). The application may combine data from product-specific testing done by the

applicant under contained growth conditions (e.g. laboratory or greenhouse trials) with data extracted from the scientific literature. Once confined field trials have been approved (these are normally limited to one hectare per site and to a maximum of five sites per province), CFIA has the authority to inspect them, as well as the records kept on them.

The information that CFIA makes available to the public regarding their approval decisions explains the basis for approval of unconfined release of a GM plant into the environment, such as the criteria to be addressed in deciding whether environmental safety is threatened, but neither the design of the experiments on which the assessment was based, nor their results, are included in the public Decision Document. Similarly, the latter describes the nutritional criteria to be met for livestock feed without presenting analytical data (CFIA b). Although they are not revealed to the public, these data are evidently collected, since the CFIA regulatory directive of July 10, 2000 reminds applicants that "experiments should generate data which can be used to address the five key criteria of environmental safety assessments" (CFIA 2000). In addition, CFIA directives indicate that statistically valid experimental designs are required for testing plants with novel traits, and that all such work is to be of the standard required for peer-reviewed research publications. In the absence of independent peer review, however, the Decision Document is in no sense equivalent to a peer-reviewed scientific paper, and in the Panel's view, the decision-making process in general lacks transparency, and thus credibility. This issue is examined further in Chapter 9 of the Report.

Despite the existence of an explicit CFIA decision framework (Figure 3.1), the Panel is of the impression that the actual decision process varies greatly from application to application. This is not necessarily an undesirable situation, since a case-by-case analysis allows the flexibility required to respond appropriately to the unique characteristics of each application. However, this degree of discretion can also make it difficult for applicants to know exactly what the approval requirements will be for their product, a problem that CFIA apparently deals with by establishing an ongoing dialogue with each applicant. This enables the Agency to comment on the application and its possible deficiencies, and to request further experimental data or information, as it deems necessary. Again, while this consultation with its advice process clearly has a positive aspect, the Panel is concerned that, without independent review, it also has the potential for allowing inappropriate decisions to be made.

Symptomatic of the lack of clarity in the current process is the ambiguous application of the principle of "substantial equivalence". Although "substantial equivalence" is explicitly mentioned in CFIA directives (see 1.2.9, Regulatory Directive 2000-07), and appears to operate as a decision threshold in the schematic representation of the decision-making process (Figure 3.1), in Panel interviews CFIA representatives claimed that it is used more as a guiding principle

than as an end point (decision threshold). The problems associated with use of "substantial equivalence" as a decision threshold are explored further in Chapter 7 of the Report.

An additional factor potentially affecting the nature of the CFIA decision process is Canada's recent (July 1998) commitment to harmonization with the US on regulation of agricultural biotechnology (CIFA c). Officials from CFIA, Health Canada and the Animal and Plant Health Inspection Service (APHIS) of the US Department of Agriculture signed an agreement on commonalities in molecular genetic characterization of transgenic products, and on the development of reviewers' checklists. An international exchange of information and harmonization of procedures is generally commendable, but it does not lessen the responsibility for thorough assessments in Canada.

Health Canada

Many GM crops are destined, as a whole or as specific parts, for the human food supply system. For this reason, they must not only obtain CFIA approval, but must also be assessed by Health Canada. Health Canada gains its jurisdiction to regulate in this area from the Food and Drugs Act and Regulations, within which GM foods come under the Novel Food Regulations. While this regulation establishes important background criteria, such as the defining of novel foods and setting the time frame for a government response, the more instructive document is that entitled *Guidelines for the Safety Assessment of Novel Foods* (Health Canada, 1994). These guidelines (as opposed to regulations) specify that a guiding principle in the safety assessment is based on a "comparison of molecular, compositional and nutritional data for the modified organism to those of its traditional counterpart". They suggest that data should be provided on dietary exposure, nutrient composition, anti-nutrients, and nutrient bioavailability. If concerns still remain following this analysis, "toxicity studies would be required as necessary, on the whole food, food constituent or specific component in question". Finally, using data supplied by the applicant, Environment Canada and Health Canada consult together to decide whether a product is "toxic" to the environment and human health (Health Canada a).

After reviewing the relevant documents and holding discussions with Health Canada personnel, it appears to the Panel that no formal criteria or decision-making framework exists for food safety approvals of GM products by Health Canada. Decisions are largely made on a case-by-case, ad hoc basis. An applicant's first contact with Health Canada usually involves an informal meeting at which the applicant may be given a sense of the type of studies to be undertaken and the information to be provided in a full application. Following this initial meeting, and perhaps several more meetings with Health Canada personnel, a full application may be submitted. The contents of this application are based loosely on, though not specifically prescribed by, the Guidelines. Health Canada must respond to this "notification" within 45 days, and then has 90

days to issue a decision. Health Canada reviews the material within 45 days and then either asks for more information, or makes a decision to approve or not to approve. As in the CFIA procedures, the applicant is responsible for supplying all of the data to be evaluated, which may be supplemented by any relevant scientific literature. No independent testing of the safety of a GM food by a governmental or other, independent, laboratory is required.

The decisions for approval of a novel food are made public by Health Canada. These documents provide the product name, the name of the proponent, the decision date and further information in a manner similar to the CFIA Decision Documents (Health Canada b). Again, the data on which the decision was based are not revealed. If an approval is issued, it could be accompanied by specific conditions, such as requiring labelling for possible allergens, because Health Canada has jurisdiction over labelling for health and safety issues.

Approvals of GM food additives, such as flavours and enzymes that are derived from GM microorganisms, are handled somewhat differently from foodstuffs themselves. They are essentially evaluated as new food additives, and the application submitted to Health Canada for approval must therefore present the taxonomy of the source microorganism, the history of the microbial strain including any use as a food, details of the novel DNA construct, and evidence for the absence of any pathogenic characteristics. Unlike approvals for transgenic organisms, the decision documents for these additives are not published. Instead, approvals are reflected solely in additions to the list of permitted food additives that appear in the *Canada Gazette*. Consistent with this approach, those enzymes permitted as food additives are listed in the Food and Drug Regulations (Table V, Division 16), but there is no indication whether they are derived from GM organisms or not. The current regulations thus treat purified products of "living modified organisms" differently from GM organisms themselves. These products also do not fall within a category of concern in the Cartagena Protocol on Biosafety (www.biodiv.org/biosafe/protocol/).

Environment Canada and Protection of the Environment

Current legislation respecting the environment includes CEPA, PCPA, parts of the Seeds Regulations (Part V) and the Feeds Act. With respect to approvals for GM organisms, the regulations call for information to be provided by the proponent about many aspects of the modified organism's biology and ecological niche, and concerning potential or actual environmental impacts of its unconfined release.

This information may be provided from published sources (historical information) or generated by the proponent through specific testing of the GM organism in question. However, the latter data, by definition, can presumably only reflect the results of studies conducted in confined holding facilities, rather than testing in the open environment. During the consultative

process that accompanies application for approval, Environment Canada/CFIA may waive specific information requirements if the proponent can provide persuasive supporting scientific arguments.

The information requirements as listed in the CEPA regulations are quite substantial. Several examples of ecological information requirements derived from sections of the CEPA Regulations are shown in Figure 3.2, along with an excerpt from the CEPA Regulations for field testing of GM microorganisms.

The Seeds Act provides CFIA with the authority to regulate the quality, testing, inspection and sale of seeds in Canada, while the Seeds Regulations (Part V) define regulatory requirements for both confined and unconfined release of plants with novel traits in Canada. According to CFIA (2000), these regulations address five key criteria for assessment of environmental safety: altered weediness potential, potential for outcrossing, altered plant pest potential, impact on non-target organisms and impact on biodiversity. As described above for CFIA, the generation of these data must use statistically valid experimental designs and protocols that meet the standards required for inclusion in peer-reviewed research publications. CFIA provides additional directives that outline conditions for confined field trials (Regulatory Directive Dir95-01) and more recent directives that amend these conditions (http://www.cfia-acia.agr.ca/english/plant/pbo/dir9501_3e.html; Oct. 27, 2000). The purposes of these directives and amendments are to define methods of reproductive isolation, including isolation distances or buffer zones, to place restrictions on post-harvest land use, to restrict the size and number of trials, and to provide improved guidelines on provision of information such as site maps.

The Pest Management Regulatory Agency (PMRA) of Health Canada is charged with the regulation of biological control agents for use in food production in Canada. An example of a recent regulatory decision for a naturally occurring viral biological control agent (for reducing codling moth damage on apple trees) may be found at the following site (http://www.hc-sc.gc.ca/pmra-arla/english/MenuPages/New_IE.html; Oct. 27, 2000). Thus far, GM control agents have not been presented to the PMRA for approval, but given the rapid advances in technology it is only a matter of time before this happens. The Panel determined through consultation with PMRA that new regulations and guidelines for GM pest control agents are presently under development.

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Figure 3.1: A schematic representation of the safety-based model for the regulation of plants*

STEP 1: FAMILIARITY

1.1 SPECIES: Has the plant species been grown or released into the environment in Canada?
IF YES, GO TO 1.2
IF NO/UNKNOWN, GO TO STEP 3

1.2 TRAIT: Is the trait similar to those already introduced into that species?
IF YES, GO TO 1.3
IF NO/UNKNOWN, GO TO STEP 3

1.3 TRAIT INTRODUCTION METHOD: Has the method been used before in that plant species?
IF YES, GO TO 1.4
IF NO/UNKNOWN, GO TO STEP 3

1.4 CULTIVATION: Will cultivation practices be similar to those previously used for this plant species in Canada?	IF YES, GO TO STEP 2 IF NO/UNKNOWN, GO TO STEP 3

STEP 2: SUBSTANTIAL EQUIVALENCE

2.1 In considering the following five criteria, and using data or sound scientific rationale, is it known that this plant will not result in altered environmental interaction compared to its counterpart(s)?	IF YES AND NON-TRANSGENIC, EXEMPT FROM SEEDS REGULATIONS, PART V[1] IF YES AND TRANSGENIC, GO TO 2.2 IF NO/UNKNOWN, GO TO STEP 3

2.1.1 Altered weediness potential
2.1.2 Gene flow to related species
2.1.3 Altered plant pest potential
2.1.4 Potential impact on non-target organisms
2.1.5 Potential impact on biodiversity

2.2 For traits introduced by rDNA methodologies, are the specific genetic elements the same as those previously approved by the CFIA in the same species?	IF YES, EXEMPT FROM SEEDS REGULATIONS, Part V[1] IF NO/UNKNOWN, GO TO STEP 3

STEP 3: ENVIRONMENTAL SAFETY ASSESSMENT BY CFIA

If acceptable risk, approve to regulate under Seeds Regulations, Part V
If unacceptable risk, approval is refused.

[1] *While an environmental safety assessment under the Seeds Regulations, Part V is not required, the plant may still be subject to regulation under other government Acts.*

Figure 3.2: CEPA Regulations dealing with the introduction of GM microorganisms into small-scale field trials

(Excerpts from: *Canada Gazette* Part II, Vol. 131, No. 5, p. 694. Canadian Environmental Protection Act: Regulations amending the new substances notification regulations. Schedule XVII: Information required in respect of microorganisms for introduction in an experimental field study)

Part 1. (f) a description of the biological and ecological characteristics of the micro-organism, including:
(i) the infectivity, pathogenicity to non-human species, toxicity and toxigenicity,
(ii) the conditions required for, and conditions that limit, survival, growth and replication,
(iii) the life cycle, where the micro-organism is not indigenous,
(iv) the resistance to antibiotics and tolerance to metals and pesticides, where the micro-organism is not indigenous,
(v) the involvement in biogeochemical cycling, where the micro-organism is not indigenous,
and
(vi) the mechanisms of dispersal of the micro-organism and modes of interaction with any dispersal agents;

and Part 1. (i) where the micro-organism is not indigenous, the dispersal by gene transfer of traits of pathogenicity to non-human species, toxigenicity and resistance to antibiotics, including a description of:
(i) the genetic basis for pathogenicity to non-human species, toxigenicity and resistance to antibiotics,
(ii) the capability to transfer genes, and
(iii) the conditions that might select for dispersal of traits of pathogenicity to non-human species, toxigenicity and resistance to antibiotics, and whether the conditions are likely to exist at the site of the experimental field study or within the range of dispersal of the micro-organism; and
(j) a description of the geographic distribution of the microorganism.

and Part 3. The following information in respect of the site of the experimental field study:
(a) the location and a map;
(b) the size;
(c) the distance to populated areas;
(d) the distance to any protected areas;
(e) a description of the geological landscape at the site and surrounding the site;
(f) a description of the biological diversity found at the site and surrounding the site, including
    (i) the identification of the endangered or threatened species, and
    (ii) where infectivity, pathogenicity to non-human species, toxicity and toxigenicity have been identified in subparagraph 1(f)(i), the identification of the receptor species;

(g) a comparison of the natural habitat of the micro-organism to the habitat at the site of the experimental field study, and the nature of the selection that may operate on the micro-organism at that site; and
(h) where the micro-organism is indigenous, data to demonstrate that it is indigenous.
4. The following information in respect of the experimental field study:
(d) a description of the procedures for transporting the micro-organism to and from the site of the experimental field study;
(e) a description of the procedures and design for the experimental field study, including
   (i) the method of application of the micro-organism,
   (ii) the quantity, frequency and duration of application of the micro-organism, and
   (iii) any activities associated with the experimental field study;
(f) a description of any procedures for monitoring the micro-organism and its ecological effects at the site of the experimental field study, during and after the experimental field study;
(g) a description of the security measures at the site of the experimental field study;
(h) a description of any contingency plans for accidental release;
(i) a description of any recommended procedures for terminating the experimental field study; and
(j) a description of any confinement procedures and biosafety conditions for the micro-organism at the site of the experimental field study, and a description of their effectiveness.
5. The following information in respect of the environmental fate of the micro-organism:
(a) a description of habitats where the micro-organism may persist or proliferate;
(b) the estimated quantities of the micro-organism in the air, water and soil at the points of introduction and the estimated population trends; and
(c) any other information on the environmental fate of the micro-organism.
6. The following information in respect of the ecological effects of the micro-organism:
(a) the involvement of the micro-organism in adverse ecological effects; and
(b) the potential of the micro-organism to have adverse environmental impacts that could affect the conservation and sustainable use of biological diversity.

4. POTENTIAL HUMAN HEALTH IMPACTS

INTRODUCTION

In this chapter, we examine the question of potential direct risks to human health that might arise from introduction of GM food products into the food supply system. These risks are generally categorized in three ways: possible creation of novel toxicants, possible shifts in the nutrient content of the food, and the possible creation of novel allergens. Each of these categories will be dealt with separately.

PART 1: TOXICANT ASSESSMENT

Assessment of the potential risk associated with a novel product intended for human consumption is routine practice internationally. This practice has given rise to an extensive body of knowledge derived from studies in laboratory animals and from studies of human exposure to chemical residues, microbiological contaminants and pharmaceutical agents, or to modifications in the concentrations of otherwise endogenously present substances. The goal of risk assessment is to inform the decision-making process in order to ensure public protection against unacceptable risks. The therapeutic use of life-saving drugs that may be associated with adverse side effects reflects the careful balance of risk and benefit. This paradigm has been broadly successful in supporting the development of regulations for protection of consumers from adverse health impacts in a very complex and chemically diverse modern society. Of particular interest to the Panel, however, is the suitability of the application of this traditional assessment paradigm for the challenges presented by food biotechnology.

Potential adverse health impacts in humans from exposure to toxicants in the food supply are expressed as a function of the probability, frequency and amount of exposure to the toxicants that are likely to occur (plus the severity of the resulting harm). The toxicological profile of the toxicant, whether endogenously produced or exogenously added to the food, is normally described through a series of well-characterized studies providing important information on the likely behaviour of the toxicant in the human body, and the biological end points most likely to be effected. The toxicological profile that results from these studies, and the dose required to achieve the effects, is then considered in the light of the expected frequency, intensity and duration of exposure to the toxicant under typical use conditions. From this analysis, an expression of anticipated risk can be developed. This model for the expression of risk for food components has been well described by the US National Academy of Sciences (1983) and is widely accepted internationally as the basis for informed decision making for a wide array of chemicals, including pesticides, therapeutic drugs and environmental contaminants.

The implementation of the risk assessment paradigm normally consists of four steps: 1) hazard identification, 2) dose–response evaluation, 3) exposure assessment and 4) risk characterization.

These have been described as follows:

1. **Hazard identification** is the determination of whether a substance, such as a constituent in food, is or is not causally linked to particular health effects. Hazard is usually determined experimentally in controlled toxicology studies with known doses or exposures to the toxicant under study. In practice, statistical considerations have resulted in the use of a "maximum tolerated dose" (MTD), the highest practical dose that can be administered, in most studies carried out in laboratory animals (Lu and Sielken, 1991). In the specific context of food safety assessment, the World Health Organization (WHO) (2000a) has defined "hazard" as a biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect.
2. **Dose–response evaluation** is the determination of the relationship between the magnitude of exposure and the probability of occurrence of the adverse effect under study. Dose–response assessment is the mechanism used to assess the potency or severity of the hazard in question. Many substances may lead to adverse effects only at high levels of exposure and may thus be considered to pose less severe hazards. Conversely, some substances may induce significant adverse effects even at very limited exposures and would thus be considered to pose a more severe hazard (e.g. classical anaphylactic responses to very low doses of an allergen).
3. **Exposure assessment** is the determination of the extent of exposure to a toxicant under a particular set of exposure circumstances. Exposure assessment includes the determination of the magnitude of the exposure, the frequency of the exposure and the duration of the exposure.
4. **Risk characterization** considers these first three factors and is often reported as a quantitative assessment of the probability of an adverse effect under defined exposure conditions. Hazard identification, dose–response assessment and exposure assessment are all essential elements of this risk assessment. In the specific context of food safety, WHO (2000a) defines "risk" as the function of the probability of an adverse health effect and the severity of that effect, resulting from a hazard in food.

Standard toxicological human health risk assessment as outlined above is science-based, but its accuracy depends on the degree of variability and uncertainty encountered in the assessment studies, which can lead to difficulty in extrapolation (SOT, 2000). Variability arises from the range of differences found within a natural population (e.g. genetic variability in sensitivity to a toxicant),

while uncertainty is generated by incomplete knowledge (e.g. inadequate gathering of data), or measurement error. Nevertheless, quantitative risk assessment related to specific chemically defined toxicants is widely used, and can address an array of end points, including cancer and other health risks, microbiological risk and certain ecological/environmental risks (Solomon et al., 1996).

On one level, the assessment of the safety of whole GM foods can be considered simply to require a comparison of the safety of the whole GM food when compared to the food or food constituent from which it is derived. Indeed, some authors have suggested that a useful and practical approach for such comparisons can be based on the concept of the "substantial equivalence" (WHO, 1995) of the whole GM food to the non-modified food already in the diet. The scientific robustness of this approach in the assessment of the risks of novel foods continues to be the subject of considerable scientific debate (WHO, 2000b; 2000c) and is extensively reviewed elsewhere in this Report (see Chapter 7). As indicated in that review, where substantial equivalence can be rigorously substantiated, toxicological assessment of the whole GM food would not be warranted. However, the Panel also concluded that, for the purposes of the safety assessment of GM foods for human consumption, "substantial equivalence" should be considered to have been achieved only if, within scientific certainty, there is equivalence in the genome, proteome and metabolome of the GM food when compared to that of the native food. In the absence of such evidence, the Panel felt that direct assessment of potential health impacts is called for, including toxicological testing. It then becomes necessary to consider whether the traditional toxicological paradigm can be applied in those cases.

Potential adverse health effects from GM food could result from over-expression of an existing protein or other toxicologically active constituent, resulting in much greater exposure to that constituent than previously encountered by humans in their diet. While exposure in this case would be to the same constituent as in the native food, and is thus likely to result in the same toxicological end point, exposure to much greater levels of the constituent in the GM food could lead to adverse health effects which could not be predicted by the absence of these effects at much lower levels of exposure to the constituent in the native food. In other words, the likelihood of a toxicological effect is very much related not only to the nature of the substance to which we are exposed, but also to the amount of exposure as well. In the general case, it would not be unusual to expect that as exposure increases, so might the adverse effects. In this scenario, the protein or metabolite in question can be subjected to the traditional toxicological evaluation, including repeat exposure feeding studies in laboratory animals conducted at a MTD, a dose which is, by design, typically hundreds to thousands of times greater than what might be encountered under actual human exposure conditions.

The Panel recognized that genetic engineering of crop plants may also result in the expression of a constituent which would not otherwise be found in the plant species, but which

does occur naturally. This phenomenon is illustrated in transgenic corn which is modified to express the *Bt* endotoxin (Cry3A), a protein which would never be found in this plant species, but which is normally expressed in the ubiquitous *Bacillus thuringiensis* microbe. In such cases, human exposure to the protein may already appear to be widespread and hence of little toxicological importance. However, the Panel noted that, because of dietary intake patterns of corn and corn products, human exposure to this protein in *Bt*-corn is predictably much greater than would otherwise be expected to occur. In the particular case of the *Bt* endotoxin, the Cry3A protein has been extensively tested for potential impacts on human health without adverse effects being reported, but whether this is true for other novel proteins intended for *de novo* expression or over-expression in crop plants is uncertain. It is also worth noting that many proteins, such as the *Bt* endotoxin, are rapidly destroyed when exposed to heat (as may occur in food processing) and are very labile under the acidic conditions of the human intestinal tract. In such cases, it can reasonably be expected that the protein would be readily broken down to toxicologically trivial components, thereby eliminating any potential concern of a classical toxicological response associated with food exposure to the native protein.

The successful application of the traditional toxicological paradigm to assessment of the health hazards that may be associated with dietary exposure to whole GM foods, or modified constituents of foods, depends entirely on our ability to identify the hazards. Where the modified constituent is a single new protein or metabolite, as discussed above, identification and testing of that constituent can be pursued within the framework of the toxicological paradigm. If, however, the hazard results from a pleiotropic response, and involves multiple changes in either protein or metabolic constituents that are not readily predicted from the genetic manipulation, the first step in the risk assessment procedure (hazard identification) seems likely to fail. Thus, while the Panel felt that the traditional toxicological paradigm could adequately assess the safety of *individual known hazards*, more complex changes in whole foods present a serious methodological challenge. GM whole foods are complex mixtures which, for reasons of nutritional balance, can be administered in feeding trials only at doses that are much more characteristic of typical human exposure. This precludes traditional safety factor considerations, “acceptable daily intake” estimations, and application of the widely accepted principles of the MTD in the design and interpretation of risk assessment studies (WHO, 1999; 2000e; 2000g).

In addition to this limitation, there is considerable uncertainty as to either the appropriate duration of studies, or the most meaningful indicators to monitor. In a consultation paper submitted to WHO (WHO, 2000a), Walker has suggested that a sub-chronic study of 90 days’ duration in rats is the minimum requirement which could address the safety of repeated consumption of a GM food in the diet. A recent WHO expert consultation (WHO, 2000g) also concluded that, where toxicology studies are deemed to be necessary, such studies should be

limited to no less than a 90-day repeat exposure study, unless proliferative or other important biological alterations indicated the need for further investigation. In contrast, the position recently adopted by the International Conference on Harmonization (ICH) on technical requirements for the safety assessment of pharmaceuticals (ICH, 1995; 1998) concluded that, where repeated-exposure studies were used for pharmaceutical safety testing, such studies should be of at least 180 days' duration. In addition, the ICH consultation noted that the determination of the need for carcinogenicity testing should include, among other things, an assessment of any evidence of pre-neoplastic lesions in the repeated-dose studies. It is noteworthy that, WHO also indicated the need for the assessment of proliferative changes (pre-neoplastic) in short-term repeat-exposure studies in order to determine the need for longer term chronic toxicity/carcinogenicity studies. However, WHO also concluded that such an assessment could be made from 90-day studies, while ICH concluded that a study of at least 180 days' duration would be required. Similarly, ICH (1997) has also indicated that the safety evaluation of biotechnology-derived pharmaceuticals should include a repeat exposure study of 180 days duration for those products for which human exposure is likely to exceed six months, an exposure scenario which would almost certainly apply for foods. Again, while the ICH position is directed at pharmaceuticals and not foods, the selection of study type and duration would appear to have a similar biological basis for both foods and pharmaceuticals to which long-term human exposure can be reasonably anticipated. The Panel noted that both the US National Research Council Report and the WHO Expert Report (WHO, 2000f) indicated that further toxicology studies, in addition to the 90-day studies described above, could be required to support the safety of transgenic foods. However, the WHO Report indicated only that the appearance of proliferative changes in the 90-day studies *might* trigger the need for further studies. The Panel was concerned that proliferative changes might not be expected to appear after only 90 days of exposure, and uncertain whether such changes, even if they did appear, provide a useful basis for triggering the need for further studies other than those directed at carcinogenicity or chronic toxicity.

In general, the Panel found that regulatory requirements related to toxicological assessment of GM food appeared to be ad hoc and provided little guidance either as to when specific studies would be required or what types of studies would be most informative. In particular, the Panel was unaware of any validated study protocols currently available to assess the safety of GM foods in their entirety (as opposed to food constituents) in a biologically and statistically meaningful manner. The Panel therefore concurs with the US National Research Council (NRC, 2000) in recommending the immediate initiation of research into the development of practical and scientifically robust approaches for the safety assessment of such foods.

Resistance Factors

A particularly controversial area in the application of gene transfer technology has been the use of marker genes which are co-introduced along with the DNA coding for the desired trait, thereby allowing confirmation that the gene transfer has been successfully completed. Historically, the most common marker genes selected (WHO, 2000c) have been those that code for resistance to herbicides or antibiotics. The concern related to the use of antibiotic resistance genes has focused on the possibility that these genes could find their way into pathogenic microbes, thereby potentially compromising the clinical efficacy of antibiotics used in human medicine or livestock production. Although this concern has been heightened by the rise in drug resistant bacteria and the declining effectiveness of many antibiotics, the Panel agrees with the position of the Royal Society (1998) that the widespread use of antibiotics as feed additives, coupled with the indiscriminate use of antibiotics in human medicine, likely poses a far greater risk for the selection of antibiotic resistant bacteria than transfer of marker genes from plants. However, in view of the availability of alternative technologies that eliminate the need to use antibiotic resistance genes as markers in transgenic plants, the Panel endorses the position already adopted by others (OECD, 2000; WHO, 2000d) on this topic and recommends that antibiotic resistance markers should not be used in any GM food intended for sale in Canada.

RECOMMENDATIONS

4.1 The Panel recommends that federal regulatory officials in Canada establish clear criteria regarding when and what types of toxicological studies are required to support the safety of novel constituents derived from transgenic plants.

4.2 The Panel recommends that regulatory authorities establish a scientific rationale that will allow the safety evaluation of whole foods derived from transgenic plants. In view of the international interest in this area, the Panel further recommends that Canadian regulatory officials collaborate with colleagues internationally to establish such a rationale and/or to sponsor the research necessary to support its development.

4.3 The Panel recommends that, in view of the availability of suitable alternative markers, antibiotic resistance markers should not be used in transgenic plants intended for human consumption.

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PART 2: THREATS TO HUMAN HEALTH FROM ALLERGENS IN GM FOODS

Food-allergic individuals and their families need to be extremely cautious about the components and ingredients of processed foods they ingest because of the risk that trace amounts of an allergenic food contaminant may cause a severe, potentially life-threatening allergic reaction (Yunginger et al., 1988; Sampson et al., 1992; Canadian Paediatric Society, 1994; Zarkadas et al., 1999). The only current treatment of food allergy is avoidance. A recent report from Montreal indicated that peanut-allergic children and their families experience considerably more impairment in their quality of life and family relations in comparison to children with chronic musculoskeletal disease, attesting to the substantial negative impact of severe food allergies (Primeau et al., 1999).

It is already difficult for food-allergic individuals to understand the different ways cross-contamination of foods can occur, as well as labelling exemptions that allow allergenic foods to remain unlabelled and pose a risk to the allergic consumer (Ham Pong and Zarkadas, 1996; Steinman, 1996; Zarkadas et al., 1999). Bock and Atkins (1989) reported that, in spite of avoidance measures, 75% of peanut-allergic children accidentally ingested peanut over a five-year period. With the widespread penetration of GMO food products in the marketplace, food-allergic people may now need to contend with another variable in deciding what foods are safe to consume (i.e. do any GM food products pose a risk for allergenicity?). The Expert Panel has tried to address this question, and to consider what measures the Canadian government and industry might take to identify the potential risks and to protect the potentially allergic consumer. Allergenicity considerations have been addressed only briefly in reports from some national expert committees on GMOs such as Britain's Royal Society and the US National Academy of Science, although a more thorough approach has been presented by FAO/WHO and US regulatory agencies, and referred to by the Institute of Food Technologists (Metcalfe et al., 1996; Royal Society, 1998; USEPA, 1999a; IFT, 2000; National Academy of Sciences, 2000; Taylor, 2000). The Canadian Food Inspection Agency (CFIA) has a paucity of published information on its procedure for allergenicity assessment on GMOs (Health Canada, 1994). In the following sections, we discuss issues regarding food allergy that may be relevant to GMOs, potential risks of allergenic GMOs, current technologies available to assess allergenicity and their limitations, and how the technology has been utilized.

Mechanisms and Allergic Responses in Food Allergy

The terms "adverse food reaction" or "sensitivity" are used to mean all types of abnormal reactions to foods, and include food allergy (hypersensitivity) and food intolerance.

Food intolerance is an adverse reaction to a food that does not involve the immune system. Examples of food intolerances include lactose intolerance, the "Chinese restaurant syndrome" caused by monosodium glutamate (MSG) sensitivity, food poisoning, caffeine-induced stimulation

and wine-induced migraine. Food allergy or food hypersensitivity, on the other hand, is an adverse immunologic reaction resulting from the ingestion, and in some cases, contact or inhalation of a food or food additive. These two terms are often used interchangeably. The most widely studied mechanism of food allergy is that mediated by immunoglobulin E (IgE), an antibody which, when exposed to an allergen, causes allergy cells in the body (mast cells and basophils) to release a variety of toxic mediators (e.g. histamine and leukotrienes) which then cause an immediate allergic reaction. An allergen is a substance, usually a protein, which causes an adverse reaction by activating immunologic mechanisms. For the remainder of this discussion, reference to an "allergic reaction" will indicate an IgE-mediated reaction (Gell and Coombs classification type 1 hypersensitivity), unless otherwise specified. Other allergic or hypersensitivity reactions to foods which do not involve IgE are usually not well understood and frequently do not have easily measurable or reliable markers to indicate the presence of an immune response. A well-known example of a non-IgE-mediated food hypersensitivity is coeliac disease (gluten-sensitive enteropathy) (Leung, 1998; Zarkadas et al., 1999).

Mediators released during an allergic reaction have a variety of effects on different tissues, and allergic reactions to ingested foods can range in severity from minor itching or skin rash, to anaphylactic shock and death. Allergic reactions to foods frequently occur within minutes of ingestion, but may occasionally be delayed for as long as four hours, and usually last less than 24 hours (Ham Pong, 1990; Zarkadas et al., 1999). Anaphylaxis is a severe, dramatic allergic reaction to a food with potential life-threatening implications. The most frequent causes of anaphylaxis-related death are upper or lower airway obstruction, and hypotensive shock (Yunginger et al., 1988; Sampson et al., 1992; Leung, 1998; Zarkadas et al., 1999).

The Increasing Problem of Food Allergies

Allergic disorders include allergic rhinitis, asthma, atopic dermatitis (atopic eczema) and food allergies, and these are now among the most common diseases in industrialized countries, with up to 30% prevalence. The incidence of allergic diseases has been estimated to have increased by 30% to 50% in the last 15 years (Kjellman, 1977; Aberg et al., 1995; Moneret-Vautrin, 1998; Habbick et al., 1999). The prevalence of food allergy in the general population varies in different studies, but ranges from 0.3% to 8% in children declining with age to 1% to 2% of adults (Leung, 1998; Zarkadas et al., 1999). Food-related anaphylaxis is felt to be rising in frequency, and one report indicated that food allergy was the cause of 34% of emergency room visits for treatment of anaphylaxis in the US (Kemp et al., 1995). There is some concern that the rising trend in general allergic disorders is also putting a higher proportion of the population at risk for development specifically of food allergies.

The Transfer of Allergens by Genetic Modification

There is no question that allergenic proteins can be transferred by genetic engineering from one organism to another. However, the current generation of GM foods approved for human consumption do not appear to have a significant potential for causing allergic reactions. In fact, there are no validated reports of allergic reactions to currently marketed GM foods as a result of the transgene protein. However, the potential risk for development of toxic or allergic reactions to GM foods will likely increase with advances in the scope and range of genetic modifications, wider acceptance of GM foods, increase in total dietary exposure to novel proteins, introduction of a greater variety of these foods, and more innovative transgenic combinations.

It is useful to review the single confirmed report of recombinant DNA technology transferring an allergenic protein to the host organism. Brazil nut allergy, like other tree nut allergies, can cause anaphylaxis, even when the nut is eaten in small amounts. Brazil nut 2S albumin storage protein was transferred into soybean to increase the content of a sulphur-containing essential amino acid, methionine, as soy is inherently methionine-deficient. The transgenic soy contained higher amounts of methionine, which could then be converted to cysteine by animals fed the soybean meal, reducing the need and cost to supplement the soybean animal feed. However, the transferred brazil nut protein made up a significant fraction, 6%, of the total protein in the soy. Initial evaluation of the brazil nut 2S albumin in a mouse model produced no evidence of allergenic potential. However, evaluation of the allergenic potential in humans by radioallergosorbent test (RAST) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by allergy prick skin test in brazil nut-allergic individuals, all showed that the major brazil nut allergen had been transferred to the soybean (Nordlee et al., 1996). This transgenic soy would therefore have posed a significant risk if ingested by brazil nut-allergic people. As a result, commercial development of this GM soybean ceased and it was never actually consumed by brazil nut-allergic individuals.

It is interesting that this brazil nut gene had been variously transferred to tobacco, bean and canola in the five years prior to discovery of its allergenic potential, without recognition of the potential risks, although none of these transgenic plants has been commercialized (Altenbach et al., 1989, 1992; Aragao et al., 1992; Saalbach et al., 1994). Similarly, a peptide encoding, in part, a portion of the melittin protein (a known allergen from honeybee venom) has been inserted into potatoes to confer bacterial and fungal resistance (Reisman et al., 1988; New Scientist, 1999), although this product has not yet been commercialized.

Transgenic combinations using donor genes from known allergenic sources carry a similar potential for transfer of the allergenic protein, if the transferred gene encodes the allergenic protein.

Potential Risks of Allergenic GM Foods

The clinical risks to consumers eating a GM food to which they are allergic range from minor to severe allergic reactions, including fatal anaphylaxis. A less obvious risk could be that, if the GM food is allergenic, and becomes incorporated as a common dietary staple or supplement, repetitive ingestion by a susceptible atopic population (i.e. genetically predisposed to produce IgE and hence develop allergies) could result in a significant number of people developing a new allergy to such a GM food. Development of an occupational allergy or asthma in food or feed handlers may also occur due to repetitive exposure by contact or inhalation of proteins.

The diagnosis of a food allergy is based, to a great extent, on an accurate history of reproducible allergic reactions resulting from challenge with the suspect food and absence of allergic reactions on avoidance of that food, followed by confirmatory allergy tests or immunologic assays for allergy. However, diagnosis reliant on history alone is confounded by the fact that, even with a suggestive history of food allergy, 60% or fewer of these subjects will have confirmed allergy on evaluation (Bock et al., 1988). A potential risk of allergenic GM foods is that a person allergic to a GM protein may not be able to identify the triggers for his or her allergic reactions if the GM protein is present in several different types of foods. It would therefore be much more difficult to pinpoint the source of the allergic reactions, since there could be several seemingly unrelated sources triggering an allergic reaction. In addition, if the GM allergen is present in a food from one grower but not another, and may be present only seasonally, the identification that a reaction may be due to the GM food is complicated by sporadic and inconsistent reactions to what appear to be the same type of food product.

It is worth noting that allergenic foods can also be GM to become hypoallergenic, as has been achieved for rice by Matsuda et al. (1996). However, in such foods even reduced levels of allergenic protein could still pose a risk for a severely allergic individual.

Food Allergens: How Much Is Too Much?

Genetic engineering to date usually involves insertion of one or a few proteins that constitute a very small fraction (usually less than 0.4%) of the total protein of the transgenic organism. It has been argued that this makes the resultant GM food unlikely to have significant adverse consequences due to the small amount of protein involved (Astwood and Fuchs, 1996b; Metcalfe et al., 1996). However, this argument is not fully valid. *Gad c 1* is a parvalbumin protein that constitutes only 0.05% to 0.1% of total cod fish muscle protein, but it is the major cod fish allergen (Bush and Hefle, 1996; Taylor and Lehrer, 1996). Therefore, even a single gene encoding for a highly allergenic protein which constitutes only a small fraction of the host organism, can be sufficient to make that organism allergenic. A food typically causes an allergic reaction on ingestion. The amounts of allergen required to cause allergic reactions can be remarkably small, so

that cross-contamination is a major concern when trying to avoid those particular foods. Peanut-allergic individuals have complained of subjective symptoms (e.g. itchy throat) during oral challenge with as little as 0.01 to 0.1 mg of peanut. As a comparison, a peanut kernel can weigh about 700 mg, and a typical serving of peanut butter is about 30 g, implying that 1/70,000 of a peanut kernel can cause minor allergic reactions (Hourihane et al., 1997a; Koetzler and Ferguson, 2000). Anaphylactic death has occurred from 60 mg of casein, the amount found in 2 to 2.5 ml of cow's milk. Anaphylaxis has been caused by 1 to 2 g of shrimp (one medium-sized shrimp is 4 g) and objective allergic reactions have been provoked by 35 to 100 mg of peanut, 6 to 12 mg of hazelnut, 0.3 ml of cow's milk, 250 mg soy protein, 1 to 4 g of fish protein, 10 mg ovalbumin (an egg allergen) and 100 to 300 mg of cottonseed in respective allergic individuals (Yman, 1995; Bush and Hefle, 1996; Taylor and Lehler, 1996).

Physical contact with an allergenic food without ingestion can cause contact hives or rash, and if accidentally introduced in the eye, can cause marked eye swelling and even anaphylaxis (Bernstein et al., 1984; Colas de Francs et al, 1991). Patients with severe food allergies have reported allergic reactions to the relevant aerosolized food (e.g. to the smell of cooking seafood, steam from cooking potatoes, and the smell of peanut in an enclosed area such as an airplane) (James et al., 1991; Eng et al., 1996; Ojoda et al., 1997; Sicherer et al., 1999). Typically, these allergic reactions to inhaled food allergens are usually minor although some of the reactions can be more severe, such as respiratory symptoms caused by the smell of peanut. Anaphylaxis is highly unlikely if the inhalant exposure is at low level, but there is at least one reported case of a fatal reaction to the smell of milk proteins (Bosetti et al., 1997). However, for other allergens such as natural rubber latex, there are many reports of anaphylaxis to aerosolized latex particles, and several instances of fatal anaphylaxis from physical contact with mucous membranes, as well as allergic reactions to latex contamination of food products due to gloves used by food handlers (Schwartz, 1995; Landwehr and Boguniewicz, 1996). These allergic reactions to low level allergen exposure highlight the contribution even a small amount of protein can make to the allergenicity of a GM food.

What Are the Most Common Food Allergens?

Nine groups of foods have been identified by an expert committee on food labelling (Agriculture and Agri-Food Canada, and Health Canada Food and Drug Regulations) as being the most likely to cause severe allergic and anaphylactic reactions in Canadians (Zarkadas et al., 1999). These foods are peanuts, tree nuts (almond, brazil nut, cashew, macadamia, hazelnut or filbert, pecan, pine nut, pistachio, walnut), cow's milk, egg, fish, shellfish (crustaceans and mollusks), soy, wheat and sesame seeds. With the exception of sesame seeds, many of these foods appear on similar lists from the UK, US and the World Health Organization Codex committee

(Hide et al., 1994; FAO/WHO, 1998; Zarkadas et al., 1999). These foods account for over 90% of the reported food allergies worldwide. However, a large number of food proteins may cause allergic reactions, and one list has documented 160 such food or food products (Hefle et al., 1996). In addition, allergies to raw fruits and vegetables causing the "Oral Allergy Syndrome", a usually mild and common type of food allergy affecting the oropharyngeal mucosa, were often not included in epidemiological studies on food allergy. These allergies to raw fruits and vegetables may in fact be the most common single group of food allergies (Pastorello and Ortolani, 1996).

Can Genetic Modification Increase the Risk of Development of Food Allergy?

The development of food and other allergies requires a complex interplay of host and environmental factors. An atopic predisposition, that is an allergic genotype, is crucial to the development of allergies, although some disorders, in particular occupational asthma, can be induced in non-atopic individuals (those who have no genetic predisposition to allergic disorders). Whether the phenotypic expression of an allergic genotype occurs depends on multiple factors. The degree of allergen exposure is important, and in some cases repetitive, prolonged and high level of exposure increases the risk of allergy. However, for allergens such as peanut, sporadic and low level exposure appears to be sufficient to promote sensitization. Total dietary exposure is important and may explain why peanut allergy is more common in North America, rice allergy in Eastern Asia especially Japan, fish allergy in Scandinavia, chickpea allergy in India, wheat allergy in America and Europe (Lehrer et al., 1996), and, on a more local level, edible "bird's nest" anaphylaxis in Singapore (Goh et al., 1999).

There is a concern that use of a transgene in a staple food, or a common transgene in several types of commonly ingested food, may increase the concentration of such a GM protein in the food stream or occupational environment and thereby increase the risk of development of allergy to that GM protein. Examples of non-GM foods introduced into the North American diet which then began to provoke allergic reactions as consumption increased include kiwi, mango, avocado, and other exotic fruits (Freye, 1989; Gall et al., 1984; Moneret-Vautrin, 1998). The same phenomenon has been occurring in Europe with the increased use of peanut as a food additive. This induction of food allergies by increasing total dietary exposure may be difficult to detect because of an initially low frequency in the population, and because years of ingestion may be required to provoke an allergic response.

The timing of introduction of an allergenic food can determine development of an allergy. Certain food allergies in children (e.g. cow's milk, wheat, soy and egg allergies) are often self-limited and disappear in early childhood, whereas allergies to peanut, tree nuts, seafood and seeds are usually lifelong (Ham Pong, 1990; Zarkadas et al., 1999). Early introduction of these and other food proteins to the infant's relatively immature immune system may encourage

development of an allergy. Infants and young children therefore appear to be more susceptible to developing food allergies, resulting in a higher incidence. Conversely, delaying introduction of these foods (e.g. by breastfeeding exclusively until age 6 months) can lower the risk of development of an allergy by bypassing the crucial stage of an infant's life when such a food allergy can be more easily induced by exposure (Host et al., 1999; AAP, 2000).

The potentially widespread use of GM food products as food additives and staple foods, including use in baby foods, may lead to earlier introduction of these novel proteins to susceptible infants either directly or via the presence of the maternally ingested proteins in breast milk. Several maternal dietary food proteins have been detected in breast milk, including bovine milk (beta-lactoglobulin), egg (ovomucoid and ovalbumin), wheat (gliadin) (Hemmings and Kulangara, 1978; Jakobsson and Lindberg et al., 1983; Cant et al., 1985; Harmatz and Bloch, 1988; Host et al., 1988), and peanut (Vadas 1999). Although controversial, there are sufficient studies to suggest that maternal avoidance of allergenic foods during breast-feeding can reduce the risk of atopic disease, in particular atopic eczema, in the breast-fed infant, and that exposure to these proteins while breast-feeding can promote allergic sensitization and allergic symptoms in the breast-fed infant (Jakobsson, 1983; Cant et al., 1985; Zeiger et al., 1986; Halkens et al., 1992; Zeiger and Heller, 1995; Chandra, 1997; Baumgartner et al., 1998; Ewan, 1998; Host et al., 1988; Vandenplas, 1998; Host et al., 1999; AAP, 2000). There is also the unconfirmed possibility that proteins from the diet of cows can contaminate cow's milk resulting in indirect exposure especially to infants and young children. Early exposure to inhalant proteins also appears to affect allergy development in susceptible infants (Korsgaard and Dahl, 1983; Businco et al., 1988). One British study reported that 80% of peanut-allergic children had allergic reactions on their first known contact with peanut, indicating that they had previously been exposed inadvertently to peanut in order to become allergic, likely by such means as described above (Hourihane and Kilburn, 1997b).

There is also some evidence to suggest that prenatal sensitization to food and inhalant allergens can occur, and that maternal dietary avoidance during pregnancy may reduce the risk of allergy development in the child. This is an even more controversial area than sensitization via breast-feeding, although there is ample evidence that the human fetus can mount immune responses to *in utero* allergens from 22 weeks of gestation (Van Asperen et al., 1983; Renz et al., 1991; Piccinni et al., 1993; Jones et al., 1996; Warner et al., 1996; Jones et al., 1998). These issues highlight the susceptibility of children to allergenic dietary proteins, the potential risks to children of allergenic proteins even if consumed mostly by adults, and the risk of inducing food allergy in the population by widespread exposure to allergenic GM proteins.

Some proteins seem to be intrinsically more allergenic than others, and different varieties of the same plant vary in their allergen contents, including peanut, avocado and wheat (Taylor and

Lehrer, 1996). Genetic engineering of food plants may have potential pleiotropic effects (collateral changes as a result of the transgene having an effect simultaneously on more than one characteristic) on the host, such as altering the intrinsic allergenicity of the protein itself (e.g. by glycosylation) or the amount of allergenic protein expressed.

The route of exposure of a food allergen can influence development of an allergy. Inhalation or frequent skin contact with certain proteins can provoke an occupational allergy or asthma, and examples of these include psyllium (a laxative derived from the husks of *Plantago solidago*), natural rubber latex, shellfish (snow crab and prawns), egg, horse dander, and grains (wheat and rye) (Chan-Yeung, 1990; James et al., 1991; Arlian et al., 1992; Anibarro et al., 1993; Kanny and Monteret-Vautrin, 1995; Witteman et al., 1995; Bush and Hefle, 1996; Fanta and Ebner, 1998; Moneret-Vautrin, 1998). Some of these occupationally sensitized workers who react to these proteins by contact or inhalation, may then develop allergic reactions when they ingest the product, as has been reported for egg, psyllium, mare's milk and natural rubber latex. The transfer of a portion of honeybee venom allergen, melittin, to potatoes (Osusky et al., 2000) raises concern that if the antigenic epitope(s) of melittin happen to be included in the transgene product, commercialization of this GM potato could sensitize consumers to honeybee venom and thus predispose them to a potentially lethal insect sting allergy.

Can We Accurately Assess or Predict the Allergenicity of a Protein?

There are well-recognized specific immunological methods for detecting the presence and quantity of known allergens in a food product. The problem arises where the donor gene and its novel protein are not known to be allergenic, in which case specific immunoreactive diagnostic reagents to assess allergenicity (specifically, IgE from humans allergic to that protein) are not available. In such cases, indirect tests have to be relied on to assess the potential for allergenicity. These indirect tests are fairly non-specific, and therefore must be interpreted with caution. There is currently no single assay or combination that will accurately predict the allergenic potential of proteins from food or non-food sources not previously identified as being allergenic in human subjects.

Nevertheless, these indirect non-immunologic tests are the only techniques currently available to assess the allergenic potential of a novel protein. Full evaluation of a novel protein should include all the steps outlined below, unless allergenicity is confirmed or strongly suspected by initial testing. The National Academy of Sciences (2000) Committee on GM pest-protected plants stated, "The strong likelihood that gene products currently found in commercial transgenic pest-protected plants are not allergens does not remove the need for a minimum of properly planned and executed tests". A novel protein that has undergone a properly designed and executed series of tests for allergenicity should be considered a low risk for allergenicity if all

results are negative (Astwood and Fuchs, 1996b; Lehrer et al., 1996; Metcalfe et al., 1996; Kimber et al., 1999; National Academy of Science, 2000; Taylor, 2000). It would be prudent to monitor for any unanticipated allergic effects following introduction of a GM food where the transgenic protein is novel to the human diet. However, the joint FAO/WHO Expert Consultation Committee (FAO/WHO, 2000) felt that observational studies would be unlikely to identify any long-term adverse effects of GM foods against the background of undesirable effects of conventional foods.

Approach to Allergenicity Assessment

The transgenic protein should be evaluated by:

- Consideration of the source from which the donor gene is derived (i.e. the donor organism)
- Comparison of the donor protein to known allergens
- *In vitro* and *in vivo* immunologic analysis to assess allergenic potential
- Assessment of key physicochemical characteristics which are common to allergenic proteins
- Prevalence of known allergy to the donor protein
- Potential changes in endogenous host allergens as a result of gene transfer (pleiotrophic effect)

Source of Donor Gene

If the donor organism has known allergenic proteins, then it must be verified whether the transferred gene has introduced allergenic proteins to the host organism by using standard immunological assays. Donor genes from allergenic non-food sources such as pollen, fungal spores, insect venom, animal dander also need to be considered, if used for gene transfer. There is ample evidence that non-food allergens, if ingested, can provoke allergic reactions. These include royal jelly (secretions of worker honeybees), bee-collected pollen, plant parts (e.g. chamomile, echinacea and psyllium), housedust and storage mites, and mould proteins in flour and lactase enzyme (Subiza et al., 1989; Erban et al., 1993; Tee et al., 1993; Florido-Lopez et al., 1995; Kanny and Moneret-Vautrin, 1995; Binkley, 1996; Thien et al., 1996; Blanco et al., 1997; Moneret-Vautrin, 1998).

Donor genes from common food sources are easiest to evaluate because there is a clear history of previous consumption, presumably with data on presence and frequency of allergic reactions, and thus some availability of specific human IgE for testing. However, if the gene comes from an exotic food source, then previous consumption history by a large portion of the population is unknown, and human IgE to the food may not be available.

The allergenicity of a large number of non-food proteins that could be potentially used in genetic engineering is essentially unknown. Insecticidal crystal protein endotoxin from *Bacillis thuringiensis* (*Bt*) is considered safe because of a 30-year history of use as a microbial insecticide with exposure to workers by mostly contact, inhalation and, to a limited extent, ingestion, although a recent report suggests that exposure may cause some immune changes (production of IgG and IgE antibodies) of unclear significance as these were not associated with clinical disease (Bernstein et al., 1999). The common use of substantial levels of this protein (and its congeners) in GM corn and potato, however, is increasing exposure by ingestion, a route not usually encountered.

Comparison with Known Allergens

Many of the known allergenic epitopes (portions of protein responsible for the immune response) are at least 8 to 12 amino acids long. There are databases of known food and non-food allergens which can be used to compare amino acid sequences of novel proteins to known allergens. If a match occurs when comparing the transgenic protein to allergens, then that protein should be considered to be potentially allergenic. The FAO (WHO) expert consultation report (Taylor, 2000) and other authors have suggested a conservative approach of an identity match of eight contiguous amino acids indicating a positive index of allergenicity, although this conservative indexing may overestimate the number of potentially allergenic novel proteins.

Of the approximately tens to hundreds of thousands of proteins that can be found in a plant, only one or two are usually major allergens (Astwood and Fuchs, 1996b; Metcalfe et al., 1996). A major allergen is defined as an allergen to which over 50% of individuals allergic to that food react by *in vivo* or *in vitro* testing. Unfortunately, the amino acid sequences for allergenic epitopes are known for only a few allergens, and expanding current databases by identifying and characterizing more food allergens is an area requiring more research. Important foods where the allergenic proteins have been characterized include peanut, cow’s milk, egg, shrimp, codfish, soybean and wheat. Examining the amino acid sequence identifies only epitopes with a common linear amino acid sequence, but some allergens derive their allergenicity by virtue of their tertiary or 3D structure and not their linear structure (Metcalfe et al., 1996). The birch pollen allergen *Bet V 3*, for instance, contains discontinuous conformational epitopes. These conformational epitopes cannot be detected as being allergenic by linear amino acid sequencing and, indeed, most antibodies produced by an allergic individual to inhalant allergens appear to be toward discontinuous epitopes, but it is unknown whether this applies to antibodies to food allergens (Taylor and Lehrer, 1996).

In Vitro ***and*** **In Vivo** ***Immunologic Analysis***

These are the most sensitive and specific tests of allergenicity. *In vitro* immunologic analysis assesses the immunochemical reactivity of the transgenic protein and requires specific IgE in sera from individuals known to be allergic to the donor protein in order to determine whether such a protein is allergenic. These assays cannot be performed if no allergic individuals are available to provide IgE for testing. If the donor protein is known to be allergenic, then these are the first tests to be performed and a positive reaction confirms allergenicity. However, occupationally exposed workers can develop IgE antibodies to a protein without clinical allergic disease, and they may then serve as a source of these antibodies for assays. Of interest, therefore, is the recent detection of IgE antibodies to *Bt* spores in some exposed farm workers since *Bt* genes have been used extensively in GMOs (Bernstein et al., 1999). Conversely, these assays can also be used to detect the presence of IgE antibodies in an individual to a GM protein in order to assess whether that person may have developed a potential allergy.

In Vitro ***Assays***

In vitro assays can detect the presence and quantity of allergenic proteins in a food, and to a certain degree whether the allergenicity of the protein has been altered. These assays include solid-phase immunoassays such as the radio allergosorbent test (RAST), enzyme-linked immunosorbent assay (ELISA), and their respective inhibition assays; immunoblot techniques (e.g. SDS-PAGE); and less commonly used for food allergy evaluation, immunoelectrophoresis and crossed radio immunoelectrophoresis. RAST and ELISA can detect the presence of the test allergen in a GM food and inhibition immunoassays are even more useful to assess both the presence and degree of allergenicity of such a protein. Inhibition assays have been used to detect traces of allergenic proteins contaminating foods, to assess the effects of processing on allergenicity of peanut and soybean-derived products, and to test the allergenicity of a transgenic soybean bio-engineered with a brazil nut gene. Health Canada (Food Research Division, Food Directorate) currently has the ability to test for peanut, egg, soy, milk and hazelnut proteins, using competitive enzyme immunoassays as part of its evaluation process for allergens contaminating processed foods (Health Canada, 2000). Rapid dipstick immunoassay kits for detection of allergenic proteins as food contaminants are being developed (Clare Mills et al., 1997). SDS-PAGE with immunoblotting is an excellent method for the separation and detection of allergens, especially where multiple allergens exist in a food.

An accurate immunologic analysis depends on the quality of the material used. Allergenic food proteins have not been standardized, and are thus subject to significant variability in quality, depending on the source. Availability of purified standardized food allergens in sufficient quantities (e.g. derived from recombinant DNA technology) would reduce this variability, and this

represents another area requiring research. Reliability of human sera from individuals allergic to the donor protein may be compromised by a) misdiagnosis — the person is not truly allergic, or b) the person may be allergic to only one or some of several possible allergens in the donor food. To overcome these drawbacks, Metcalfe and colleagues (1996) proposed an approach developed by the International Food Biotechnology Council and the Allergy and Immunology Institute of the International Life Sciences Institute (IFT, 2000). They have suggested that serum donors should meet rigid criteria for diagnosis of an allergy (clear-cut, convincing, severe allergic-type reaction to isolated ingestion of that food), and/or positive double-blind, placebo-controlled oral food challenge (DBPCFC) (Bock, 1980; Bock et al., 1988). These authors also suggested that sera from at least 14 such allergic individuals should be used for *in vitro* assays, for appropriate reliability. If these give negative results, they suggest going on to *in vivo* assays such as allergy prick skin test, and if necessary, DBPCFC.

One potential drawback of current guidelines for allergenicity assessment of donor proteins from known allergenic sources is the usual presence of multiple allergens in a particular food source, examples of which include peanut, soy, egg and cow's milk. When 14 test sera are used to assess allergenicity of a GM food, and all are negative, this provides greater than 99.9% assurance that a major allergen from the donor organism has not been transferred, and greater than 95% assurance that a minor allergen affecting at least 20% of the sensitive population has not been transferred (Metcalfe et al., 1996; Taylor, 2000). This would leave a small number of food allergic individuals who are allergic only to minor food allergens at risk if that transgenic food is declared "non-allergenic" on the above statistical basis. However, it is worth emphasizing that a minor food allergen (an allergen to which less than 50% of individuals allergic to that food are allergic) is just as capable of causing severe reactions as a major allergen.

A review of brazil nut-allergic test subjects used to assess allergenicity of a transgenic soybean containing brazil nut allergens showed that one of the nine test subjects was allergic only to a minor brazil nut allergen (Nordlee et al., 1996). If only a minor allergen from a host source is transferred to a GM food, and the frequency of allergy specifically and solely to the minor allergen is less than 20% of individuals allergic to the host food, then there is some chance, albeit low, that the battery of sera used will not contain IgE to that minor allergen. In such a case, immunological assays may not detect the presence of the minor allergen in the GM food, and other steps in the evaluation process will have to be carried out.

In Vivo *Studies*

Further analysis of allergenicity can be performed using human subjects known to be allergic to the donor protein. This consists of allergy prick skin testing using suitable concentrations of extracts of the host food, the native donor food, and the GM food. These

extracts are pricked into the epidermal layers of the allergic individual's skin, and observed for a localized allergic reaction consisting of a hive at the test site within 15 minutes. This reaction indicates that the test subject's immune system has identified that particular food as carrying an allergen against which the subject has previously developed IgE antibodies. If desired, these volunteers can be further evaluated by DBPCFC with the GM food to confirm the presence or absence of that particular allergenic protein in the GM food. The confirmation of the presence of an allergen by DBPCFC is the most reliable method, but often is not practical because it requires the physical presence of human volunteers. DBPCFC will most likely be necessary for the final evaluation of a GM food containing a gene from a known allergenic source when all previous evaluations show no indication of allergenicity.

The detection of specific IgE antibodies in an individual does not necessarily mean the presence of a clinical allergy. Other factors may also determine whether an allergic reaction occurs, and those with IgE antibodies but no symptoms on exposure to the relevant allergen have "asymptomatic sensitivity". In the case of food allergy, only 30% to 40% of individuals with IgE antibodies to foods will have allergic reactions on ingesting the food (Bock et al., 1988; Sampson, 1988). However, high levels of IgE antibodies do increase the probability of a clinical allergy.

The reliability of prick skin testing is clearly affected by the quality of the food allergen extract used. Prick skin tests to some foods, especially fruits and vegetables, must be performed with freshly prepared extracts due to the labile nature of the allergenic protein. Improper extraction of the food proteins may lead to inadequate concentrations of relevant allergens in the skin test extract, which results in false negative tests. Processing, heating or digestion of a protein can destroy protein antigenicity, but it can also enhance the allergenicity by formation of new epitopes or neonantigens. In these cases, allergy testing with the native food may also produce false negative results, which may explain some case reports of allergic reactions to sesame seeds without demonstrable IgE sensitization (Eberlein-Konig et al., 1995). This emphasizes the need in selected cases for food challenges (DBPCFC).

Animal models for *in vivo* testing may be useful in certain circumstances but there is no animal model currently able to predict accurately human allergic responses and therefore donor protein allergenicity. Examples of current animal models for allergy evaluation include mouse models to evaluate IgE responses to recombinant allergens, and guinea pig and rat models of anaphylaxis. The difficulties in using animal models to assess allergic potential have been documented (Metcalfe et al., 1996; Taylor and Lehrer, 1996; Kimber et al., 1999). An appropriate animal model of food allergy must be such that the test animal is able to mount a human-type IgE antibody response to foods under near natural conditions; that is, it must be able to mount a significant IgE antibody response to the food allergen in question, by the usual provocation route, oral exposure. At the same time, it must be able to tolerate the majority of food proteins. In

addition, the animal's allergic responses should be similar to those seen in humans, be consistent and easily reproducible. Unfortunately, no such model exists. Current animal models mount IgE responses only with difficulty, and under abnormal conditions such as induction by injection of the allergen together with adjuvant to enhance the immune response. Even in these models, the IgE responsiveness can vary at different times under the same conditions.

Use of animal models to screen for potential immunogenicity (ability to mount an immune response by producing IgG antibodies) and using this response as an indicator of potential allergenicity or ability to induce IgE has met with some significant failures with respect to human foods. Guinea pig and rabbit animal models were used to assess the allergenicity of partially hydrolysed cow's milk whey formulas. These animal models predicted reduced immunogenicity of the whey hydrolysate formulas which were then marketed as "hypoallergenic", but in fact they remained sufficiently allergenic to cause reactions in most cow's milk-allergic infants (Palud et al., 1985; Taylor and Lehrer, 1996; Host et al., 1999; AAP, 2000). Assessment of the brazil nut albumin protein in transgenic soybean for potential allergenicity using a mouse model of passive cutaneous anaphylaxis did not elicit an allergic or immune response, leading to the erroneous conclusion that there was no allergenic protein transfer to the soybean (Astwood and Fuchs,1996b; Nordlee et al., 1996).

More reliable animal models mounting human-type IgE responses as described above have the potential to reduce the present dependence on human sera. These could be developed through standard breeding and selection, or perhaps even transgenically. Kleiner et al. (1999) and Li et al. (2000) have recently developed mouse models of cow's milk allergy and peanut allergy.

Physicochemical Characteristics

Protein allergens tend to have certain characteristics such as molecular weight between 10 and 70 kiloDaltons (kDa), and resistance to acid and proteolytic enzyme digestion (i.e. resistance to gastric digestion). They are usually proteins, often glycosylated (sugar compounds attached to the protein), are relatively stable to heating, have acid isoelectric points, are often water-soluble albumins or salt-soluble globulins, and usually make up a significant percent (1% to 80%) of the protein content of the source material (Matsuda and Nakamura, 1993; Astwood and Fuchs, 1996b; Bush and Heffle, 1996; Metcalfe et al., 1996). GM food proteins showing diagnostic physicochemical characteristics of allergens such as molecular weight, and stability to heat and gastric digestion may then be considered to have a higher potential for allergenicity, if direct immunologic assays are not available.

However, these are not particularly reliable indicators. There are a number of heat-labile or partially heat-labile food proteins which denature and lose conformational epitopes on heating, such as cow's milk whey beta-lactoglobulin and bovine serum albumin, chicken egg ovomucoid,

rice glutenin and globulin, soy glycinin and some peanut proteins (Matsuda and Nakamura, 1993; Bush and Hefle, 1996; Taylor and Lehrer, 1996). Similarly, while food allergens often constitute a significant portion of a food's proteins, as mentioned previously, the potency of an allergen may compensate for its relative paucity in a food, and some major allergens such as codfish *Gad c 1* are present only in small proportions of the food. Likewise, some protein allergens are low molecular weight such as the 9 kDa plant lipid transfer proteins (LTP) which are important allergens of the Prunoideae family which include peaches, plums and cherries (Breiteneder and Ebner, 2000; Rodriguez et al., 2000); LTP from barley used in beer foam formation (Curioni et al., 1999); and the 8 kDa soybean hull protein responsible for asthma outbreaks in Spain (Gonzalez et al., 1991). Interestingly, heating some allergenic proteins may actually increase their allergenicity, in some instances by chemical glycosylation (Maillard reaction). Examples include cow's milk beta-lactoglobulin, pecan, fish, shrimp, snow crab and limpet (Malanin et al.,, 1995; Berrens, 1996; Taylor and Lehrer, 1996; Moneret-Vautrin, 1998).

It should be noted that some allergenic compounds are not proteins. Known examples are shrimp transfer RNA, inulin (a carbohydrate); and vegetable gums such as carrageenan and tragacanth (Danoff et al., 1978; Yeates, 1991; Tarlo et al., 1995; Bush and Hefle, 1996; Gay-Crosier et al., 2000). In addition, a large number of foods, in particular raw fruits, raw vegetables and spices, have heat-labile proteins (e.g. chitinases and *Bet v 1*) which cause the Oral Allergy Syndrome. All these exceptions would not be identified as allergens by their physicochemical criteria, or by any other criteria if they were present as a novel protein GM.

Prevalence of Allergy to the Donor Protein

If the prevalence of an allergy to the donor protein is very low, then it may not be recognized or it may be very difficult to obtain sufficient amounts of sera from allergic persons to adequately test for the presence of allergens. Limited testing can lead to missed allergenic characteristics. Metcalfe and colleagues (1996) and Taylor (2000) have suggested that a lower standard of assurance of absence of allergen transfer be used where limited human sera is available for testing. However, this limitation could be overcome by establishing a registry and/or a bank of serum from allergic people.

Potential Changes in Host Allergenicity

When a host organism is being genetically engineered, it must be ensured that the modified host organism has not undergone pleiotropic changes that result in creation of novel allergens. These effects could include the GMO being induced to express higher levels of its own endogenous allergens beyond what might be expected from natural variability; or endogenous or transgenic proteins undergoing post-translational modification including glycosylation or alteration of its 3D structure, perhaps changing allergenicity or creating new allergens. These possibilities can be assessed using the *in vitro* immunological assays described above. Such changes could increase the severity of an allergy reaction in persons already allergic to the host or donor food, and increase total dietary exposure to a more allergenic food.

Genetic engineering may affect endogenous allergen content in several ways, including altering the host plant metabolic pathways and enhancing allergen production. Storage, and effect of plant hormones such as ethylene, are known to increase the allergenicity of foods such as apple, banana and peach. Stress may also increase the levels of allergenic proteins (e.g. *Bet v 1*) in some fruits and vegetables (Hsieh et al., 1995; Pastorello and Ortolani, 1996; Breiteneder and Ebner, 2000; Rodriguez et al., 2000; Sanchez-Monge et al., 2000). Different varieties of foods such as peanut, avocado and wheat vary in their allergen content (Bush and Hefle, 1996) and these levels could conceivably be altered further by genetic modification.

Other Considerations in Allergenicity Assessment

Some factors may affect results of allergenicity evaluation, and may need to be considered in assessing or designing studies. The detection of clinically important food allergens may be complicated by the presence of cross-reactive allergens in the food. These cross-reactive allergens may show some similarity to the test allergenic protein and may give positive results, usually weakly, using *in vivo* and *in vitro* immunologic assays, but may not cause true allergic reactions. Botanically related plants may have cross-reactive allergens, examples of which are legumes such as peanut, peas, beans and soy. Thus, a peanut-allergic person may have IgE antibodies to peas but can eat peas without allergic reactions. Clusters of allergens also occur when distinct, non-botanically related plants and foods share similar allergens, as in the birch/celery/spice syndrome, otherwise known as the Oral Allergy Syndrome, where individuals allergic to *Bet v 1*, the major allergen of birch pollen, also have allergic reactions to homologous pathogenesis-related proteins found in certain fruits, nuts, vegetables and spices (Halmepuro et al., 1984; Breiteneder and Ebner, 2000; Rodriguez et al., 2000). Another important food allergy cluster is the latex-fruit syndrome due to an allergy to *Hev b 2*, a pathogen-induced endoglucanase enzyme found in natural rubber latex but found also in avocado, banana, chestnut and kiwi (Moller et al., 1998; Breiteneder and Ebner, 2000).

Certain homologous proteins are also found in widely varying species such as tropomyosin in shrimp, chicken, mosquito, cockroach and housedust mites, although the cross-reactivity is probably low (Bush and Hefle, 1996). The degree of sequence homology is important. The major shrimp allergen, tropomyosin, is a protein present in many other foods including beef, pork and chicken with which it shares 60% sequence homology, but beef, pork and chicken tropomyosin are rarely allergenic (Lehrer et al., 1996). In these cases, a definitive answer regarding allergenicity can only be based on specific IgE-based assays.

Some food allergies, such as those involved in the Oral Allergy Syndrome, produce allergenic effects on the oral mucosa during mastication of the food. Oral challenges with these foods by swallowing (e.g. in capsule form as is the preferred method for DBPCFC rather than chewing) may not reproduce the allergic reactions seen in the real-life process of chewing and eating. Foods normally eaten cooked but which may occasionally be handled or eaten raw (e.g. potato) may show a different allergenicity profile depending on how the food is presented for oral challenge. The converse situation also needs to be considered (i.e. foods which increase their allergenicity on heating).

If an allergen is expressed in the host organism, the site of expression such as in the leaves, pollen or edible part of the plant is important in considering risk. There would be different implications if the allergen is not expressed in the edible but rather in the non-edible portion of the plant, or if the allergenic plant proteins might be inhaled during processing of plant parts with the accompanying risk of occupational sensitization.

An Example of the Evaluation Process to Assess Allergenicity

A useful model to examine is the evaluation process used by the US Environmental Protection Agency (USEPA) for potential allergenicity of the *Cry9C* gene from *Bt* encoding for an insecticidal crystal protein endotoxin, inserted into GM corn, using the criteria described above (USEPA, 1999a, b). The USEPA used an approach developed during the 1994 Interagency Conference on potential allergenicity in transgenic food crops, which included the USEPA, Food and Drug Administration, and Department of Agriculture (Fox, 1994; USEPA, 1999a). They evaluated the source of the donor gene, which had no known allergenic history despite 30 years of use as a microbial insecticide. However, *Bt* is not a food product, and this particular BT gene had been modified and therefore had a much shorter exposure history. Comparison with known allergens showed no epitope sequence homology and therefore no resemblance. Immunologic analysis was limited by the absence of material from humans clinically allergic to BT since none has been identified. A brown Norway rat model of IgE immune response was inconclusive although an immune response was provoked. Physicochemical characteristics showed that the *Cry9C* protein did show some stability to simulated gastric fluid digestion and some heat stability.

In addition, its molecular weight of 68.7 kDa fell at the upper end of the range for allergen molecular weight. These physicochemical characteristics suggested a potential for allergenicity, although it was present at a low level (0.17% of total weight), features not usually seen with important allergens. Potential pleiotropic effects were examined by screening the genetically altered *Cry9C* corn with serum from suspected corn-reactive subjects, which did not detect any alterations in the intrinsic allergenic status of the GM corn.

On the basis of two positive biochemical characteristics found in allergens (relative stability to heat and gastric digestion), the USEPA declined to upgrade *Cry9C Bt* corn for use as human food in 1999 and left unchanged the approval as an animal feed and for industrial use. Other *Bt* genes encoding similar endotoxins (e.g. *Cry1A* and *Cry3A*) have been approved for human consumption in GM corn and potato as they did not demonstrate the biochemical characteristics shown by the *Cry9C* gene product, nor did they exhibit any other signs to indicate potential allergenicity (USEPA, 1995).

Unfortunately, the *Cry9C* corn (termed StarLink (TM)) inadvertently contaminated corn destined for human use resulting in a large recall of corn-derived food products in the US in October 2000. In addition, *Cry9C* protein was discovered in some non-StarLink seed corn, and although this was felt to be due to physical contamination, cross pollination from StarLink corn could not be ruled out as the source. The accidental introduction of StarLink corn into the human food chain prompted a further review of the potential allergenicity of *Cry9C*, and of mechanisms for assessing suspected allergenic reactions to StarLink corn. This review was conducted by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP), the primary scientific peer review mechanism of the USEPA (FIFRA, 2000; US EPA, 2000a).

FIFRA-SAP concluded that the *Cry9C* protein had a medium likelihood of proving to be a potential allergen. They considered that at least 7 of 34 complaints regarding reactions to a corn-containing meal were probably allergic, based on a careful assessment of the history which was compatible with a food allergy, plus opportunity for exposure to the suspected protein, as well as confounding factors (e.g. other allergens). In the final analysis, the determination of whether an allergic reaction occurred to the *Cry9C* protein in StarLink corn would have to be based on detection of the *Cry9C* protein in the ingested food, detection of antibodies, especially IgE to *Cry9C* protein in the subjects' serum, and if necessary oral food challenge (DBPCFC).

At the strong urging of the USEPA, StarLink corn was voluntarily withdrawn from agricultural use, and all known existing corn contaminated with it has been restricted for non-human food use (USEPA, 2000b). Unaccounted StarLink corn could continue to enter the food supply over the next few years, however. This incident underscored the difficulty of restricting a GM food for animal/industrial use when almost indistinguishable non-GM food counterparts are simultaneously available for human consumption. It also highlights the issue of mandatory

labelling (discussed in Chapter 9, Part 2). Post-introduction surveillance of a GM food which has medium to high-risk allergenic potential is essential. This medium- to high-risk scenario must be accompanied by appropriate labelling to identify allergic reactions rapidly and accurately. However, where the risk of allergenicity is low, the justification for labelling diminishes from a scientific viewpoint, bearing in mind that the lack of labelling can lead to delayed recognition of the emergence of an allergy, with consequent under-reporting. Therefore, especially when labelling is not required, there should be mechanisms to record, evaluate and fully investigate complaints of suspected allergy, as recommended above for StarLink corn by FIFRA-SAP. In addition, since there may be potential exposure to multiple unlabelled GM proteins from different sources, evaluation of the subjects may have to include a battery of dietary GM proteins.

It could be argued that requiring GM proteins and GM foods to undergo rigorous assessment for allergenicity prior to approval as a food product, with or without labelling, represents a double standard since this process is not required when a novel or exotic non-GM food is introduced. An exotic food may still pose a risk because while there is some history of previous consumption, it may not have been extensive or monitored sufficiently to ensure its safety, and the genetic susceptibility to allergies in its native area may differ. On the other hand, the presence of an exotic or novel food in the diet is more easily identifiable, avoidable, and more easily monitored for the possibility of adverse reactions, compared to a transgenic protein in a food which may not be easily monitored as regards degree and frequency of exposure, or even whether exposure has occurred at all.

Notwithstanding the limits of current technology, a GM food which has undergone a thorough, scientifically valid evaluation process for allergenicity, with negative results, should be considered at low risk to provoke or induce allergic responses and could possibly even be safer than a non-GM novel or exotic food which has not been subjected to the same scrutiny. This evaluation process can significantly minimize allergenicity concerns and perhaps reduce the chances of GM products being used as scapegoats for a variety of real or perceived illnesses. It stands to reason that any GM food with potential or identified allergenicity must either not be approved for human consumption, or if approved, then it must be appropriately labelled.

Summary

The identification of potential allergens in GMOs is accurate and reliable when assessing transgenes from known allergenic sources. It is indirect and non-specific with respect to novel proteins from sources not known to be allergenic and without a history of extensive human exposure. Even for the nine identified major food allergens responsible for most of the severe allergic reactions to foods in Canadians, only some of the allergens have been chemically characterized, and none has been standardized. *In vivo* and *in vitro* techniques are available to

assess accurately and reliably potential allergenicity when dealing with proteins from known allergenic sources. Where the donor gene comes from an organism not known to be allergenic, or of unknown allergenicity (e.g. an exotic food, or a product not normally ingested as food), assessment becomes more difficult. There is currently no single assay or combination of assays that will accurately predict the allergenic potential of protein from sources not known to be allergenic. Nevertheless, using an array of properly designed and executed assays, and knowledge regarding the characteristics of the transgene, a GM food may then be considered relatively safe for allergic consumers and comparable to its non-GM counterpart, if all tests are negative. Not withstanding negative allergenicity assessments, however, if the transgene is derived from a source of unknown allergenicity, post-introduction surveillance may be prudent to monitor for any unanticipated allergic effects, recognizing that this may be more difficult without corresponding labelling of GM foods.

RECOMMENDATIONS

4.4 The Panel recommends that the Canadian government should support research initiatives to increase the reliability, accuracy and sensitivity of current methodology to assess allergenicity of a food protein, as well as efforts to develop new technologies to assist in these assessments. This would include further research into the identification, purification, characterization and standardization of common food allergens, as well as their respective antibodies (e.g. monoclonal animal antibodies) which can be used in detection systems; development of reliable animal models of human-type IgE antibody responses; identification of specific characteristics which can accurately and specifically identify a novel protein as being allergenic; and development of rapid assays (e.g. dipstick-type assays) for use by food processors and consumers to detect allergenic contaminants.

4.5 The Panel recommends the strengthening of infrastructures, and where none exists, development of these infrastructures to facilitate evaluation of the allergenicity of GM proteins. This could include development of a central bank of serum from properly screened individuals allergic to proteins which might be used for genetic engineering, a pool of standardized food allergens and the novel GM food proteins or the GM food extracts, maintenance and updating of allergen sequence databases, and a registry of food-allergic volunteers. These would enhance the ability of government agencies such as the Canadian Food Inspection Agency to broaden the scope of and its technological ability to detect allergenic proteins.

4.6 The Panel recommends development of mechanisms for after-market surveillance of GM foods incorporating a novel protein, if there are data to indicate its effectiveness, to detect the emergence of consumers developing allergies to such a food either through increase in total dietary exposure over the long term, or occurrence of unanticipated and unpredicted allergic reactions. This could include a central reporting registry and/or epidemiological studies to assess changes in frequency, pattern and clinical presentations of allergy-related complaints. The infrastructure in Recommendation 4.5 could be used to verify scientifically reports of allergic reactions and detect emergence of allergies to GM proteins.

4.7 The Panel recommends that the appropriate government regulatory agencies have in place a specific, scientifically based, comprehensive approach for ensuring that adequate allergenicity assessment will be performed on a GM food, utilizing currently available techniques combined with currently available knowledge of the characteristics of the GM protein relevant to potential allergenicity, and updating testing requirements in keeping with new technologies. Any decision not to complete a full and comprehensive allergenicity assessment should be made only after

careful consideration of the scientific rationale to support that omission. The decision to approve or not approve introduction of a GM food and the need for labelling should therefore be based on a rigorous scientific rationale.

4.8 The Panel recommends that approvals should not be given for GM products with human food counterparts that carry restrictions on their use for non-food purposes (e.g. crops approved for animal feed but not for human food). Unless there are reliable ways to guarantee the segregation and recall if necessary of these products, they should be approved only if acceptable for human consumption. If a GM food is found to have acquired additional allergenic properties from gene transfer, then that GM food should either not be marketed, or be properly labelled if marketed.

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PART 3. NUTRITION ISSUES

Introduction

A central concern in any modification of traditional food sources must be the impact of such changes on the nutrient content of the food. The components generally considered under this rubric are the content of carbohydrates (simple and complex), proteins and their constituent amino acids, fats and their fatty acid profiles, vitamins, dietary fibre and anti-nutrients. No crop species, in itself, provides an appropriately balanced range of nutrients for human or animal consumers. A diet that effectively meets metabolic needs, therefore, must be derived from multiple sources that complement each other's nutrient strengths and deficiencies. Nutritionists, dietitians and food specialists work with databases such as the Canadian Nutrient File, which are designed to reflect the average amounts of nutrients in individual foods. Based on such data, which are derived from the history of the commercially grown varieties as a food source, a common crop (e.g. potatoes or corn) would be expected to provide certain nutrients within a known range. If the concentration of a particular nutrient happened to fall at or beyond the extremes of the range, there could be health implications, particularly for humans who rely heavily on that foodstuff in their diet.

Another nutritional parameter that normally attracts regulatory attention is the level of specific anti-nutrients in some foodstuffs. While the definition of an anti-nutrient remains unclear, the term generally refers to plant secondary metabolites that appear to have deleterious effects over time on animal or human consumers. Examples of anti-nutrients whose levels are usually monitored in new Canadian crop varieties include erucic acid and glucosinolates in canola, cyanogenic glycosides in flax, and glycoalkaloids in potatoes.

Impacts of Genetic Engineering

Genetic engineering of common crops in Canada has thus far not focused on nutrient modification, and any impacts on the major nutrients in these first generation GM crops would presumably have to be the result of pleiotropic effects of the transgene(s). To date, GM foods produced from approved GM crops have been judged to be nutritionally equivalent to their non-GM counterparts, presumably on the basis of chemical analyses for the classes of nutrients described above. However, the Panel is unaware of any public data available for confirmation of this assumption. In fact, the only nutritional information related to GM foods available to the public appears in the Decision Documents released by the CFIA for animal feeds (CFIA, Plant Biotechnology Decision Documents, at: www.cfia-acia.ca/english/plaveg/pbo/isda01_.html). This is restricted to a statement about proximate analysis (an unsophisticated procedure that analyzes the material for crude protein, crude fat, ash and moisture levels) and in some cases examines certain groups of amino acids, together with the comment that anti-nutrients did not exceed acceptable levels.

New GM varieties specifically designed to present altered profiles of fatty acids, altered starch qualities, and/or altered protein profiles are all currently under development. Some of the proposed changes have the potential to improve the foodstuff's nutritional quality, such as GM corn whose storage proteins contain an enhanced level of lysine, the limiting amino acid in that food. Other nutritional modifications being explored include increased vitamin content (e.g. carotenoids, a source of vitamin A – as in "golden rice"), higher iron content, and enhanced concentrations of nutraceuticals such as lignans and bioflavonoids (antioxidants). This ability to fortify traditional foodstuffs is expected to be marketed as a direct consumer benefit of GM foods. While positive impacts can be envisioned, any substantial alteration of food nutrient profiles has potential ramifications that would appear to call for careful monitoring and public reporting.

Testing

Chemical analysis provides the first level of assessment of possible changes in nutrient content in novel foods. Very powerful methodologies are now available for analysis of protein, fatty acid and carbohydrate profiles, as well as scanning for changes in secondary metabolite profiles (see Chapter 7). Nevertheless, food is a complex material with many potential interactions among its components that would be hard to predict simply by scoring the individual chemical classes. Where significant deviations from the profile ranges expected for a food component are detected, it may therefore be desirable to conduct whole organism tests designed to assess nutrient bioavailability. This is analogous to the need, discussed earlier in this chapter, to determine whether novel foods bring with them any new toxicological risks.

The assessment could involve either animal testing, where a suitable animal model has been developed, or testing in human subjects. A foodstuff could be tested as part of a diet fed to experimental animals, which could then be monitored for health and growth over their normal lifetime. Where chemical analysis has detected changes in particular food components, it may be more useful to examine the impacts of those changes by using the specific component as a dietary ingredient. Proteins have been evaluated in this fashion in experimental animals for at least four decades (Campbell and Chapman, 1959). In the early tests done in rats, protein was 10% by weight of the diet and the duration of the feeding was four weeks. A faster method, which gives similar results and involves using an amino acid profile corrected for the digestibility of the protein, took no more than two weeks (Sarwar and McDonough, 1990). This method has been adopted by the FAO/WHO Expert Consultation (1991) on protein quality evaluation. Suitable tests should be available for evaluating specific fat and/or carbohydrate compositions, while testing the impacts of discrete anti-nutrients would follow the well-established protocols developed for toxicological testing.

Human foods differ from animal feeds in that the emphasis is less on rapid weight gain in a

shortened life span, or on enhanced milk production, and more on a long, healthy life as free as possible from disease. Over the many decades of human life, foods are expected to provide all recognized nutritional requirements. Relatively short-term animal tests may yield valuable information, but establishing the impacts of long-term ingestion of a food would involve the systematic monitoring of human populations. This issue is clearly related to the question of labelling of GM foods, and has been explored in Chapter 9 of this Report.

RECOMMENDATIONS

4.9 The Panel recommends that all assessments of GM foods, which compare the test material with an appropriate control, should meet the standards necessary for publication in a peer-reviewed journal, and all information relative to the assessment should be available for public scrutiny. The data should include the full nutrient composition (Health Canada, 1994), an analysis of any anti-nutrient, and where applicable, a protein evaluation such as that approved by FAO.

4.10 The Panel recommends that protocols should be developed for the testing of future GE foods in experimental diets.

4.11 The Panel recommends that the Canadian Nutrient File should be updated to include the composition of GE foods and be readily available to the public.

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5. CONSIDERATIONS IN THE USE OF BIOTECHNOLOGY IN ANIMAL PRODUCTION SYSTEMS

INTRODUCTION

The Canadian regulatory bodies, food producers and processors, and Canadian consumers have experience with food from GM microbes and crops. However, food-producing animals, including fish, differ from bacteria and plants in so many respects that the development and promotion of biotechnology will be substantially different. This chapter will address the animal welfare, food safety and environmental issues related to biotechnology applications in animal production systems, either through the development of a transgenic animal or the use of products derived through biotechnology in animal production systems.

PART 1. GENETICALLY MODIFIED ANIMALS

Modifications in transgenic animals may induce undesirable changes in an animal's physiology and behaviour. This could lead, for example, to an altered level of production of a natural or genetically altered protein that increases the susceptibility to disease.

Potential Threats to Animal Health and Welfare

Fish

Comparatively little information is available on the effects of transgenesis relative to fish health and welfare. Existing documentation has concentrated, for the most part, on deleterious consequences to fish morphology, respiratory capacity, and locomotion associated with the introduction of growth hormone (GH) gene constructs in some transgenic variants of salmonids, notably Pacific and Atlantic salmon.

Nonetheless, despite the relative paucity of data, it seems clear that pleiotropy (unintended genetically based changes to an organism's phenotype associated with the introduced gene construct) associated with the introduction of novel gene constructs is the *rule* rather than the exception in fish. This pleiotropy has been manifested by changes to enzyme activity, gross anatomy, behaviour and, in all likelihood, hormonal activity. The following sections reflect the current status of knowledge relative to impact of transgenesis on fish health and welfare.

Changes in muscle cellularity, muscle enzyme activity and gene expression

Transgenesis has been reported to affect the muscle cellularity and muscle enzyme activity in coho salmon (*Oncorhynchus kisutch*) containing a GH gene construct (Hill et al., 2000). The levels of activity of two enzymes in the white muscle— phosphofructokinase and cytochrome

oxidase — were 275% and 31% higher, respectively, in transgenic fish. This finding is consistent with the hypothesis that the muscle of transgenic fish has greater glycolytic and aerobic requirements than the muscle of non-transgenic fish.

There also is evidence that insertion of single gene constructs can affect the activity of non-targeted genes. Increased gene expression is suggested by elevated levels of transcription (ribosomal proteins and tRNA) and changes in muscle ultrastructure (myosin heavy chain and skeletal α-actin) in transgenic coho salmon relative to their non-transgenic counterparts (Hill et al., 2000). From a human health perspective, the same research documented an increase in the amount of the Ca^{2+} transport protein, parvalbumin-β, in transgenic coho, a protein that has been identified as a major food allergen in fish (Lindstom et al., 1996).

Changes in gross anatomy

Growth hormone gene constructs can cause significant morphological deformities in fish. For example, Devlin et al. (1995a) have documented morphological abnormalities among transgenic coho salmon in the cranial, jaw and opercular regions. From an animal health perspective, these morphological abnormalities affected the ability of transgenic fish to feed properly and to irrigate their gills at a level that would permit normal rates of respiration. Similar changes to body shape have been observed in transgenic carp (*Cyprinus carpio*) (Chen et al., 1993; Dunham and Devlin, 1999) and in non-transgenic channel catfish (*Ictalurus punctatus*) injected with growth hormone (Dunham et al., 1992). Cartilage overgrowth in the cranial and opercular regions has also been associated with increased mortality among the progeny of transgenic coho salmon, again because of their inability to feed normally or to irrigate their gills properly (Devlin et al., 1995b). However, the incidence of such abnormalities can be expected to decrease with selection for transgenic broodstock that produce a reduced range of the phenotypic variability manifested by novel gene constructs.

In addition to these changes to the head region, transgenesis can affect the overall shape of transgenic fish. Ostenfeld et al. (1998) reported that insertion of the pOnMTGH1 gene construct into coho salmon significantly altered the shape and allometry of affected fish. McLean et al. (1997) have suggested that the reduced swimming ability reported for transgenic coho (Farrell et al., 1997) may be attributed in part to changes in skin and pressure drag effected by these changes to body shape.

Changes to swimming ability and foraging behaviour

Transformation with a GH gene construct has been reported to affect the swimming behaviour of salmonids. Farrell et al. (1997) found the critical swimming speeds of growth-enhanced transgenic coho salmon to be significantly lower than those of non-transgenic controls

of the same size and same age. These reduced swimming speeds in transgenic coho may be caused by ontogenetic delay or from disruption of the locomotor muscles and [or] their associated respiratory, circulatory and nervous systems (Farrell et al., 1997). Despite this example of reduced swimming speed in GH-enhanced fish, it is not clear that such an effect is a general one. Such reductions, for example, have not been observed between transgenic Atlantic salmon and non-transgenic controls (Abrahams and Sutterlin, 1999).

Increases in overall activity are apparent from simple observation of transgenic salmonids into which a GH gene construct has been introduced. This increased activity appears to be associated with increased feeding rate (Abrahams and Sutterlin, 1999; Devlin et al., 1999) and speed of movement (Abrahams and Sutterlin, 1999). One consequence of this increased activity appears to be reduced vigilance to predators (Abrahams and Sutterlin, 1999), an observation that has also been made in non-transgenic, GH-treated salmonids (Jönsson et al., 1996 a, b).

Other pleiotropic effects

To date, the dominant form of genetic manipulation undertaken for the aquaculture industry has involved growth hormone gene constructs. As suggested by the morphological and enzymatic changes described above, the consequences of increased levels of GH are unlikely to be restricted to increases in growth rate alone.

The growth-promoting effects of GH are achieved in part through the activity of insulin-like growth factor I (IGF-I), a substance produced by the liver and peripheral cells to promote mitosis and/or differentiation of fibroblasts, prechondrocytes and other cells critical to the development of new skeletal and cartilaginous tissue (Goodman, 1993). In addition to the direct effects of GH on the metabolism of target cells in adipose, liver, muscle and pancreatic tissue, GH also can have indirect effects that may affect the health of transgenic fish. For example, Goodman (1993) reported that GH can modify the sensitivity of cells to, as well as production of, other hormones, such as insulin and catecholamines. Indeed, Mori and Devlin (1999) have reported 50% to 83% reductions in the size of the pituitary gland of transgenic coho salmon relative to non-transgenic controls, although it is not known if such changes affect the activity of hormones other than those associated with growth.

Farm Animals

Transgenic research in support of animal agriculture for food production lags behind the progress made with fish, but will undergo a revolution due to the explosive growth of molecular-based technologies being driven by supporting research platforms. Perhaps the most notable is the recent development of methods of somatic cell nuclear transfer and the production of clones from these somatic cells for livestock species (McCreath et al., 2000). This advance overcomes the

serious limitations of pronuclear micro-injection for the production of GM livestock species (Polejaeva and Campbell, 2000).

Over the next 5 to 10 years, we should see many of the advances required for development and commercial application of GM germplasm and transgenic animals in dairy cattle, swine, and some poultry. To this end, much of the research and development will be driven by corporate strategies to capture the potential economic value of transgenic technology for improved growth rate and carcass composition in meat-producing animals and compositional modification of milk and eggs. Another critical requirement to realize commercial application, particularly for recalcitrant traits like fertility and disease resistance, is the opening of the genetic "black box", which is currently taking place as a result of rapid integration of genomics analysis technologies in research on all livestock species (Gellin et al., 2000). Once the information (i.e. identity of genomic regions that encode quantitative trait loci of economic importance) and technologies (e.g. cell culture-based transgenesis) are finally in place, there is little doubt that breeding companies will offer animals bred from proprietary germplasm. Such animals may have traits conferring enhanced production efficiency, or in some way meet consumer demand by, for example, offering improved nutritional value.

Another potential application of transgenic technology in livestock production is to increase the safety of animal products for human consumption through strategies to increase disease resistance and thus reduce reliance on antibiotics. Opportunities exist for genetic modifications that reduce product susceptibility to spoilage or bacterial contamination. The recent demonstration in mice, using a gene knockout strategy, of the inactivation of the prion gene involved in transmissible spongiform encephalopathies (TSE), reveals the possibility that similar genetic modifications may be achieved in livestock species (i.e. to prevent scrapie in sheep and BSE or "mad cow disease" in cattle) to reduce their susceptibility to diseases (Flechsig et al., 2000).

Research efforts in transgenic animals can be categorized into two general areas; the first being production of proteins to modify the normal functioning in the animal (e.g. modification of fat or protein synthesis by the mammary gland, transfer of growth hormone genes into pigs, transfer of cysteine synthesis genes in sheep for enhanced wool production); the second being production of a target protein that is not part of normal animal function (e.g. spider silk production by goats) which may be for food, pharmaceutical or industrial production purposes. The following sections extract information from the published literature relevant to animal health and welfare in order that an understanding of the scope of this issue can be provided.

Changes in muscle cellularity, muscle enzyme activity and gene expression

Production of excess growth hormone in transgenic pigs carrying various growth hormone transgenes (Pursel and Rexroad, 1993) caused multiple physiological effects, including reduced fat carcass, alterations of muscle fibres, thickening of the skin and redistribution of major carcass components, but did not result in "giantism" as was observed in growth hormone-enhanced GM mice (Palmiter et al., 1982). Many of these same effects are not observed in pigs given daily injections of PST.

The carcass fat in transgenic pigs expressing either a bovine, ovine, or human growth hormone gene construct was reduced 84%, 82% and 62 %, respectively, compared to sibling control pigs in a recent study reported by Solomon et al. (1997). Pursel et al. (1996) suggested that the dramatic reductions in carcass fat are related to an interference in insulin's ability to stimulate lipogenesis, even though insulin was 20-fold higher in the transgenic pig than in the control sibling. As well as reduced carcass fat, major decreases in carcass fatty acid levels have been observed in transgenic pigs, decreases of 70% to 87% in saturated fats, 69% to 89% in monounsaturated fatty acids and 36% to 71% for polyunsaturated fatty acids. The impact of these and other genetic modifications on animal health and welfare, or on food safety, has received little research attention to date.

Pursel et al. (1999) attempted to achieve the same objective by transferring a gene construct that consisted of an avian α-skeletal actin promoter attached to human insulin-like growth factor I into swine. Insulin-like growth factor-I mediates many of the same effects as growth hormone without the dramatic effect on the systemic physiology of the animal. However, the variable response to the transgene in individual animals is apparent. For example, in this study three of 14 transgenic animals died of endocarditis or cardiac hemorrhage, ages ranging from preweaning to just before first parturition. Cause of death may have been associated with the expression of the IGF-I transgene in the cardiac muscle, indicating that control of expression in various tissues will need to be evaluated, not just for individual animals, but also at various physiological stages of life.

Reproductive efficiencies continue to limit progress in development of transgenic animals. Only a small proportion of reconstructed embryos develop to become live offspring. Success with lambs varied from 0.04% with adult cells to 1.7% for fetal-derived cells (Wilmut et al., 1997). Even when considering only the proportion of embryos that became live lambs, the proportion ranged between 3.4% and 7.5 %. There may also be complications at the time of birth. A number of lambs derived by nuclear transfer in the work conducted by Wilmut et al. (1997) died at birth due to congenital abnormalities in the cardiovascular or urinogenital systems. Other problems encountered include large birth weights (perhaps related to culture conditions for the zygote), increased gestation length, immature lung development at birth and slow onset of labour.

These results inevitably trigger major animal welfare concerns and require full consideration prior to release of the technology. While some of the identified problems will be overcome with technology improvements, there will also be situations in which the allowable impact on animal welfare may need careful definition (e.g. the number of times an animal is subjected to Caesarean section in its lifetime).

Increased incidence of mutations and other pleiotropic effects

Current technologies used in the development of transgenic animals have improved control of insertion sites of the construct gene, but examples are accumulating of transgene instability and unexpected patterns of gene expression in transgenic animals. In many cases, the insertional mutation is recessive and is not expressed until subsequent generations. Again, movement of the technology into the commercial arena will require informed debate and decision regarding whether there is an acceptable rate of increase for mutation, and whether any unexpected pattern of gene expression is acceptable.

The biological complexity of animals, the longer generation time and our reduced ability to select for desirable traits in transgenic animals will all delay our ability to quantitatively and qualitatively assess the impact of this technology on the health and welfare of the individual animal or of farm livestock populations as a whole. Assessing the animal welfare advantages (reduced killing of surplus male chicks or castration of males) or disadvantages associated with production systems using GM animals is difficult because there is no consistency of response among transgenic animals at this early stage of technology development. In addition, adverse effects may be identified only when the animals are challenged, or may only be apparent during one stage of the animal's development. This emphasizes the importance of studying animal welfare and health as an ongoing part of further technological development and monitoring this throughout the life of the transgenic animal.

Altered nutritional and welfare needs of transgenic animals

Genetic manipulations usually have as their target the production of proteins that influence specific metabolic pathways. These alterations can impact on the animal's inability to synthesize specific enzyme substrates or co-factors. That kind of alteration can change the optimal balance of nutrients required by the animal, and may even alter the requirement for essential nutrients. In traditional animal selection programs, these changes and the resulting dietary or management adjustments are made over an extended period of time and are based on a reasonable working knowledge of the biochemical pathways affected by the selection process.

Suitable facilities and environmental requirements for management of genetically engineered animals will need to be considered prior to release into the commercial agriculture

sector, since normal coping mechanisms relative to the animal's physical and social environment may have been compromised. Similarly, nutritional requirements under normal and stressed conditions need to be determined. The current well-developed science behind modern animal nutrition and management should allow appropriate responses to be devised to meet the novel needs of transgenic animals, once these needs are identified and characterized.

Creation/Strengthening of Animal Commodification

Biotechnology applications in animal populations can occur for both domesticated and non-domestic species. For example, animal sensitivity to the environment could be reduced in order to allow increased nutrient resource allocation to production, or to protect animals from disease. Non-domesticated populations (e.g. Red Deer or Wapiti) could be genetically engineered for increased production of antler velvet or a pharmacological compound in antler velvet. However, as Heap (1995, as reported by Mench, 1999) pointed out in an address to the Royal Society of Agriculture, "Programmes which threaten an animal's characteristics and form by restricting its ability to reproduce normally, or which may in the future diminish its behaviour or cognition to improve productivity would raise serious intrinsic objections because of their assault on an animal's essential nature." Nevertheless, there remains a grey area as to where animal welfare issues begin and ethical issues end, relative to animal management and use, and this uncertainty will be exacerbated by introduction of transgenic technologies. Decisions are, therefore, urgently required regarding the future purpose of the technology. Animal health and welfare (as defined in the glossary) are considered in the process of product approval; however, the mandate of this Panel does not allow it to deal specifically with the ethical issues of technology application in animal production systems.

Reservoirs of Pathogens or Antibiotic-resistant Microflora

Development of animal breeds resistant to a disease would be expected to reduce the short-term requirement for vaccines and medicines. However, creation of resistance to the pathogenic effects of disease agents without blocking infection and continued dissemination of the disease agents (i.e. the animal does not exhibit symptoms but continues to be a carrier) could create additional problems concerning disease epidemiology and control, transmission to other species (including humans) and disease agent mutation (Cunningham, 1999). For example, beef and dairy cattle are currently major reservoirs of enterohemorrhagic *Escherichia coli* O157.H7 (Shere, 1998). This pathogen is an important cause of food and water contamination, leading to several hundred deaths and thousands of serious illnesses every year in North America. Although this pathogen and related enterohemorrhagic *E. coli* have co-evolved with humans over the past 4 to 5 million years (Reid, 2000), their incidence has increased over the past two decades as a

consequence of changes in farm management practices and increasing encroachment of urban areas and urban water supplies on rural farmland (Shere, 1998; Gagliardi, 2000). Conceivably, changes in farm management practices as a result of biotechnological innovations could increase animal population densities, or alter their ability to act as reservoirs, leading to further increases in the incidences of such pathogens. This risk factor should be investigated during the development of transgenic animal biotechnology products.

Loss of Animal Genetic Resources

Loss of livestock breeds has become an issue in many parts of the developed world where intensive animal agriculture systems require animal uniformity and production efficiencies to maximize economic return (Patterson, 2000). The extent of genetic variation within breeds of livestock influences the rate of genetic progress by selection and the success of genetic resource conservation in the long term. The sequencing of entire animal genomes and identification of single nucleotide polymorphisms in the genomes of agricultural species will provide a better understanding and a more complete characterization of genetic variability at the nucleotide level. However, more accurate selection techniques, allowing production and evaluation of individual animals at an early age, *in utero*, or even before fertilization in the case of artificial insemination, has the potential to erode existing genetic diversity in our farm animal populations. On the other hand, molecular biology will advance the ability to accurately assess existing genetic variation and could thereby contribute to its preservation of diversity.

Currently, many animal breed associations, and the government, maintain active registries of pedigreed animals in Canada. This has proven to be a useful tool in maintaining the integrity of registered pure breeds or populations of animals. The meat production industry is also engaged in discussions that would allow tracking of individual animals from birth to market as a means of assessing animal management and genetics in terms of final product quality and safety. There is likely to be interest on the part of both the industry and the consumer to maintain similar programs for GM animals, once they enter commercial production systems.

RECOMMENDATIONS

5.1 The Panel recommends that the Canadian Food Inspection Agency (CFIA) develop detailed guidelines describing the approval process for transgenic animals intended for (a) food production or (b) other non-food uses. Furthermore, the Panel recommends that CFIA encourage work with the Canadian Council on Animal Care (CCAC) to engage the scientific community in the development of appropriate scientific criteria for assessment of behavioural or physiological changes in animals resulting from genetic modification. (It is anticipated that applications for GM animals will occur within the next 10 years. It would be advisable to develop the decision process and criteria for each step of the process. The process could then be challenged with a test case.)

5.2 The Panel recommends that the approval process for transgenic animals include a rigorous assessment of potential impacts on three main areas: 1) the impact of the genetic modifications on animal health and welfare; 2) an environmental assessment that incorporates impacts on genetic diversity and sustainability; and 3) the human health implications of producing disease-resistant animals or those with altered metabolism (e.g. immune function). Any negative effects on animal health and welfare and the environment would require justification on the basis of significant benefit to human health or food safety.

5.3 The Panel recommends that the tracking of transgenic animals be done in a manner similar to that already in place for pedigree animals, and that registration be compulsory.

5.4 The Panel recommends that transgenic animals, and products from those animals, that have been produced for non-food purposes (e.g. the production of pharmaceuticals) not be allowed to enter the food chain unless it has been demonstrated scientifically that they are safe for human consumption.

5.5 The Panel recommends that federal and provincial governments ensure adequate public investment in university-based genomic research and education so that Canada has the capacity for independent evaluation and development of transgenic technologies.

5.6 The Panel recommends that the use of biotechnology to select superior animals be balanced with appropriate programs to maintain genetic diversity which could be threatened as a result of intensive selection pressure.

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PART 2. GENETICALLY MODIFIED FEEDS, FEED ADDITIVES AND METABOLIC MODIFIERS ADMINISTERED TO FOOD-PRODUCING ANIMALS

Biotechnology is already widely used in animal production and we can expect an increase in this activity in the future. The primary goals are to influence the nutrition of the animal, improve animal health, modify product characteristics, improve product quality or reduce adverse environmental impacts of animal agriculture. Examples of biotechnology-derived products currently in use include silage inoculants, amino acid supplements, feed enzymes, and pre- and pro-biotics. Information relative to the range of products of biotechnology being studied relative to animal health, nutrition and physiology is found in a review by Bonneau and Laarveld (1998). There continues to be interest in the application of biotechnology for production of metabolic modifiers to improve growth and lactation, feed efficiency and animal product composition. However, there are no such products registered currently for use in Canada. Interest in this area is due to the use of products such as recombinant bovine growth hormone in countries that are trading partners with Canada.

As the acreage of GM crops increases in Canada, a higher portion of the feed ingredients used in livestock production systems will consist of the resulting grains, forages, meals and by-products. To date, biotechnology applications have focused on improving agronomic characteristics of crops and the quality characteristics required for human food. Improved feeding value of GM crops for animal use is possible but has not been emphasized, mostly because animal feed (grains and oilseed) is often suitable for human consumption or is a by-product of food harvest or processing. This trend is likely to change because movement to targeted crop production for specific types and classes of animal has the potential to reduce animal production costs. The following section identifies some of the potential novel threats associated with the use of GM plants, microorganisms and pharmaceutical products in animal production.

Potential Novel Threats to Food Quality and Safety

Commercial development of feed additives and metabolic modifiers for use in animal production may involve genetic engineering. In many cases, these products (i.e. recombinant GH or IGF-I analogs produced in bacteria) have known benefits on production efficiencies, animal product quality or animal health, but more research is required to determine the potential for negative impacts in commercial settings.

Biotechnology has been responsible for a number of changes in practice relative to the use of vaccines. Issues of biosafety relative to the injection into animal tissues of naked DNA constructs coding for foreign antigen, driven by eukaryotic gene promoters that may be destined for human consumption, are still being explored.

Early technologies rendering GM microbes antibiotic resistant presented the threat of resistance transfer, especially in the animal gut. Although resistance transfer may not be a health threat to the animal, the presence of antibiotic-resistant bacteria in the human food chain is an unnecessary threat to human health.

There are two other areas of potential concern that to date have not yet been addressed to any great extent by the research community. First, there is the potential transmission of toxins from feeds derived from GM plants to the animal, and ultimately into animal products. Plants cohabit with a range of epiphytic micro flora. With any new practice, the epiphytic micro floral populations can change and potentiate toxin production. This is also true for GM plants and, therefore, some monitoring is required. Genetic transformation of plants may have an impact on patterns of gene expression. The resulting changes in the plant's composition, physiology or morphology will influence the populations and species of micro flora associated with the plant and may thereby lead to the introduction of new, or previously less common, toxins into the animal's diet. The issue extends to consideration of the behaviour of these altered microbial populations under a range of harvest and storage conditions, and the associated potential for introduction of toxins into animal diets.

A second potential concern focuses on the use of feed additives, digestion enhancers or vaccines against infectious diseases of the gastrointestinal tract. These are designed to improve digestion and gut health, often through the manipulation of gut microflora. Coupled with the diverse range of management conditions to which livestock across Canada are subjected, there may be situations in which such manipulation can cause adverse changes in gut micro floral populations relative to shedding of pathogenic organisms, with the potential contamination of animal products and ground water.

Potential Novel Threats to Animal Health or Welfare

Metabolic Enhancers

Bovine GH (also known as BST, bovine somatotropin) was the first product derived through genetic engineering to be used for modification of animal metabolism. It affects regulation of growth and lactation in cattle. A summary of experiments using genetically engineered BST showed that its administration across a range of doses increases milk production by 10% to 20%, with little effect on milk composition (Bauman, 1999; Etherton and Bauman, 1998). Half the increase in milk yield can be accounted for by an increase in efficiency from the spreading of the maintenance requirement across a larger output. Concerns regarding animal health and safety have focused on the potential for increased incidence of metabolic disease (i.e. ketosis) in the early stages of lactation, compromised immune function (i.e. increased incidence of mammary gland infections), and reduced animal longevity. However, trials conducted to date indicate that the

occurrence of these problems is similar to that seen in dairy cows at equal levels of milk production that have not received BST. This suggests that the negative impacts are associated with increased milk production, rather than with the BST itself.

Administration of high doses of recombinant porcine somatotropin to growing pigs has been shown to have adverse effects on animal health, including an increased incidence of stomach ulcers and leg problems associated with osteochondrosis and cartilage soundness (Sejrsen et al., 1996). Radical changes in the composition of tissue fatty acid profiles or shifts in lean tissue to skeletal tissue growth may enhance meat product quality but they also have the potential to increase the animal's susceptibility to infectious agents or metabolic disease.

Advancement in genetic engineering, in the case of metabolic modifiers, has resulted in the development of products with pharmacological properties that are incompletely understood. In this situation, thorough study of the new product(s) as well as the technology by which it is produced is required for assessment relative to animal health and welfare and food safety.

Vaccines

Sub-unit vaccines, pathogen attenuation by gene deletion, live vectoring of antigen by insertion of foreign antigen into gene-deleted mutants, and development of "new generation" adjuvants are all processes that have opened the door for new delivery systems for vaccines, for enhanced protection against specific pathogens, and for distinguishing between vaccinated and naturally infected animals. Concerns still being addressed within this technology envelope include consistency of the resulting immune response.

Immunomodulation of growth and lactation can be envisioned as an alternative to direct genetic manipulation of the production or response functions in transgenic animals, and it may be considered more acceptable than exogenous administration of growth or lactation promotants because the need for repeated injection is eliminated. However, this form of permanent modification of the animal's hormone production pattern is not as well understood and accepted as the promotant approach, and requires further consideration relative to both animal welfare and food quality and safety (Mepham and Forbes, 1995).

Microbially Derived Feed Supplements and Additives

Some of the first GM feeds used by livestock were "single cell" protein products used to replace plant or milk proteins in pre-ruminant and baby pig diets. Crystalline amino acids (e.g. lysine, threonine and tryptophan) are used extensively as supplements in animal diets today. Future developments may include ruminally protected amino acids and use of specific amino acid supplements as stimulants for hormone release (Hurson et al., 1995), or for gut and immune system development in young animals (Gardiner et al., 1995). Microbial enzymes are currently

being used to increase the digestibility of nutrients in feeds either in the animal gut (i.e. phytase) or during feed storage and processing to supplement host endogenous enzymes (i.e. protease and amylase), to remove toxins and anti-nutritional factors in feed ingredients (i.e. enzymes to destroy trypsin inhibitors) and to increase digestibility of the non-starch polysaccharides (i.e. ß-glucanase). Manipulation of the gut micro flora to promote growth of beneficial bacteria and/or competitively exclude pathogens can be accomplished through manipulation of the diet composition, or by inclusion of specific microflora, with the goal of improving absorption of nutrients through improved gut health. Many of these amino acids, enzymes, pre- and pro-biotics are produced by fermentation, often with GM organisms. Inclusion of GM-derived proteins in animal diets has not been reported to create novel threats to animal health or welfare. Specific research to investigate potential food or feed safety problems does not appear in the literature.

Live, GM bacteria and their products can be used in feed harvest, storage and processing. For example, GM *Lactobacillus* sp. is used in silage production to control fermentation. Although these organisms were specifically designed to be competitive in the silo environment, there has been concern that accidental release, either at the time of application or from silo seepage, could create an environmental risk if natural populations are modified. To date, use of GM microbes in the production of animal feeds has not been reported to create novel threats to the environment, although the extent of investigation is very limited.

The introduction of the tetracycline-resistant Tc^R a gene into *Prevotella ruminicola* was the first successful transfer of a gene into rumen bacteria (Flint et al., 1988). Since then, gene transfer has been used to introduce cellulase activity into a number of hind-gut bacteria to enhance acid tolerance in cellulolytic rumen bacteria, to improve protein (essential amino acid) yield by rumen bacteria, and to induce hydrogen scavenging in rumen bacteria and thereby reduce methanogenesis. Novel threats to animal health and welfare may result from microbial population shifts that could, for example, cause a reduced capability of gut microflora to adapt to dietary changes.

The current limitation to this technology rests with the GM organism's ability to compete in the natural rumen or hind-gut environment. Gregg et al. (1993) did report rumen survival for a 50-day period for a GM strain of *Bacteriodes fibrisolvens* in which the added genetic material did not provide any known competitive advantage.

Transduction and conjugation are well-known mechanisms of transfer of genetic material between microorganisms. The probability of gene transfer in the gastrointestinal tract is dependent on the nature of the GM microbe and the characteristics of the gene construct. A transfer gene that enhances the survival characteristics of the recipient microorganism might provide phage resistance, virulence, adherence, substrate utilization or production of bacterial antibiotics, and could impact animal health and food safety.

No reports of gene transfers from ingested plant or microbial DNA into the epithelial cells of animals have been found, with the exception of genes from infectious agents such as viral DNA. It is assumed that even if such a transfer were to occur, the transformed epithelial cells would not be maintained, because of the continuous replacement of these cell. Further investigation of these assumptions is warranted as the range and source of new gene constructs in the animal gut increase, and as gut cell metabolism is altered due to animal feeding or genetic manipulations.

The potential now exists to replace many of the microbially derived feed additives with plants that are GM to directly enhance the animal's feed supply. For example, incorporation of phytase in crops would improve phosphorus availability to the animal, as opposed to the current process of supplementing animal diets with recombinant phytase enzyme derived from GM microbes.

To date, no animal health or production problems have been reported to result from to the use of GM grains or oilseeds in feed preparations. Feed industry representatives have reported that introduced gene constructs that reduce the plant's susceptibility to pests (e.g. *Bt* corn) also result in a significant reduction of mycotoxins in the plant material (Lobo, 2000), probably due to improved overall plant health. Research is being conducted to test these field observations. Advances in the production of crops that more adequately meet the nutritional needs of the animal may actually reduce the industry demand for dietary additives such as enzymes and amino acids currently derived from GM microbes.

Novel problems related to the production of GM crops for animal consumption would centre around the issue of increased storage and handling capacity requirements at the feed mill. For example, a high-fat grain may be advantageous in poultry diets, but could cause digestive problems if inadvertently added to a ruminant diet. The plant–microbe interactions that lead to mycotoxin production are also still poorly understood. In general, improved plant health will result in less colonization by problem fungi, but certain changes in the plant's biochemical make-up and morphology may also change the pattern of microbial colonization, thus potentiating conditions for previously undetermined toxin production, or increased mycotoxin production even with similar colonization.

Potential Threats from Concentration of GM Products in the Animal's Food Stream

Increased production of GM microflora and plants for food production can lead to increased opportunities to use GM-derived byproducts as animal feeds. Byproducts may be unused plant parts (e.g. stem and leaf material following crop harvest), unprocessed plant products that do not meet standards for human use (e.g. immature, high mycotoxin levels), byproducts associated with food or industrial processing, or restaurant waste. The potential for

concentration of unidentified anti-nutritive factors or toxins in the byproducts of processing, therefore, needs to be addressed.

With the exception of vegetables, all plants with novel traits approved by the Canadian federal government prior to March 1999 have been approved for animal feed as well as human food (Barrett, 1999). However, upon review of the Supplement to the Decision Document that accompanies the approval of GM plants currently grown in Canada, it was not clear to the Panel that all plant parts are considered in the evaluation process. The potential therefore exists that plant parts destined for feed in animal production systems may not have been specifically tested in that context.

Recommendations

5.7 The Panel recommends that a national research program be established to monitor the long-term effects of GM organisms on the environment, human health, and animal health and welfare. In particular, plant–microbe interactions that could result in increased exposure to toxins in feed or food, and microbial–animal interactions that could increase exposure to human pathogens in food and water need to be studied.

5.8 The Panel recommends that changes in susceptibility of genetically engineered plants to toxin-producing microbes, and the potential transfer of these to the animal and the food supply, be evaluated as part of the approval process.

5.9 The Panel recommends that a data bank listing nutrient profiles of all GM plants that potentially can be used as animal feeds be established and maintained by the federal government.

5.10 The Panel recommends that university laboratories be involved in the validation of the safety and efficacy of GM plants and animals.

5.11 The Panel recommends that Environment Canada and the Canadian Food Inspection Agency establish an assessment process and monitoring system to ensure safe introductions of GM organisms into Canada, according to the intent of the Canadian Environmental Protection Act.

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6. ENVIRONMENTAL RISKS

INTRODUCTION

Many of the concerns surrounding recent developments in agricultural biotechnology centre on the potential ecological effects of these new varieties of organisms, and on their potential for changing agricultural practices that may, in turn, impact on the farm and hinterland environments. The following chapter is divided into four sections that review the science, the developing technologies and the potential environmental concerns for GM microbial, plant and animal (insect and fish) varieties. The Panel has focused its review on those aspects of the biology of GM organisms that are of particular concern with respect to potential environmental risks. For each taxon, where appropriate, the Panel refers to the above regulations or guidelines and we make both research and regulatory recommendations that we believe will strengthen Canada's environmental protection standards in this area.

PART 1: MICROORGANISMS IN BIOTECHNOLOGY AND THE ENVIRONMENT

No complex life forms exist in isolation from the microbial world. All plants and animals have associated microflora that form commensal, symbiotic, parasitic or pathogenic relationships with their hosts. The vast majority of these relationships benefit both the host species and the microbe, or are selectively neutral in their effects; therefore, we tend to ignore these organisms. Much less common are microbial parasites or pathogens that harm their host, and to which we pay special attention. The communities of microorganisms associated with higher life forms are invariably highly diverse, including representative species of metazoans, protozoans, fungi, algae, bacteria and archaea. The relationships of animals and plants with the microorganisms that surround them or grow within them are the result of millions of years of natural selection, operating on both the host and the associated microflora. These organisms are constantly responding to changes in the physiology and behaviour of one another as variants arise through normal evolutionary processes. Change is continual and multifaceted.

Two important concepts will be described in the next paragraphs that help to place the discussion of potential environmental effects of transgenic organisms into the context of state-of-the-art research in microbial ecology. These concepts are:

- the microbial species concept
- the diversity of microorganisms in the natural environment.

These have been singled out to emphasize the difficulty of making predictions about the effects of transgenic biotechnology products on the microbial environment. Nevertheless, knowledge is growing in this field and we can point out areas of potential concern.

The Microbial Species Concept

This concept is central to discussions of how transgenic organisms might affect microorganisms, microbial communities, the processes they carry out and the ecosystems that depend upon their activity. It is also central to discussions of gene transfer, either from higher plants and animals to their associated microflora, or from microbial biotechnology products to other microorganisms in the surrounding environment. The definition of what constitutes a "species" in bacteria is not well developed. Phenotypic differences (in structure, biochemistry or physiology) between species formed the basis for bacterial identification throughout most of the history of the discipline. In the past 30 years, molecular methods of describing species have been developed. In the past two decades, these molecular methods have been integrated with phenotypic methods to yield a "polyphasic" approach to species identification. There is an ongoing explosion in microbial diversity research that is pushing its way ever further into extreme environments and into the common soil and aquatic habitats of the biosphere (Olsen et al., 1994; Hugenholtz et al., 1998; Whitman et al., 1998). The microbial phylogenetic tree is becoming more and more branched and subdivided and, as a result, the genus and species concept in microbiology is rapidly coming to signify an arbitrarily defined section of the phylogenetic tree. For example, an assumption used by some microbiologists is that a ribosomal RNA (16S rRNA, a commonly used phylogenetic character) sequence difference greater than 2% between two organisms indicates that these are two different species. This definition presents some problems, especially within certain taxonomic groups such as the Proteobacteria where new species are rapidly filling all of the gaps between the branches of the phylogenetic tree. In these highly populated taxonomic groups, a continuum of species variants flows one into another. Because we know most about groups such as the Proteobacteria, they have become a focus of the biotechnology industry. They also represent major colonizers of plant and animal epithelial surfaces, and they are the taxa that contribute many of the harmful bacteria associated with disease.

The Diversity of Microorganisms in the Natural Environment

Microbial diversity is greater, by ecological, phenotypic and genetic measures, than that of any other taxonomic group (Olsen et al., 1994; Tiedje, 1994). Soils are arguably the most complex habitats within the biosphere. They contain a large proportion of the estimated 10^{30} microbial cells in the biosphere (Whitman et al., 1998). The soil environment is the focus of many concerns associated with the potential environmental effects of transgenic plants and animals. Soils contain enormous numbers of microbial species, although the measured number depends both on how the measurement is carried out and, as explained above, how one defines a microbial species. The number of species in a gram of typical agricultural or forest soils from temperate

regions has been estimated to be from thousands to tens of thousands (Torsvik et al., 1990; Ovreas and Torsvik, 1998).

In many natural habitats such as soils, aquatic sediments and marine environments, between 0.01% and 1% of microbial species are currently culturable using standard methods. That means that only a very small sample of microorganisms can actually be studied under laboratory conditions as pure cultures. Limitations of culture methods can be demonstrated by employing culture-independent methods to detect the DNA associated with the uncultured fraction of microorganisms (Hugenholtz et al., 1998). For example, a recent study of grassland soil diversity was conducted by cloning and sequencing of 16S rRNA genes amplified by PCR directly from soil-extracted DNA and comparing these sequences to 16S rRNA genes of over 600 cultured species from the same soil (Felske et al., 1999). The results showed that there was no correlation between the culture collection and the 16SrRNA clone library. This does not mean that the uncultured species can never be cultured in the laboratory and characterized; rather, it reflects limitations in our culture methods and in the resources we have at hand to study the vast diversity of microorganisms that occur in these habitats. As a consequence, we cannot reduce complex microbial communities and their function to a set of known biotic and abiotic interactions. We are beginning to appreciate the fact that we have just scratched the surface in understanding microbial diversity in terms of the numbers of species, their relative abundance, and the differences that exist in diversity between different ecozones (Borneman and Triplett, 1997) or even between different patches in outwardly uniform-looking agricultural soils (Siciliano and Germida, 1999). For the foreseeable future, there must be many unknowns concerning the details of microbial community function in most natural habitats. Despite this limitation, some experimental data exist and certain predictions are possible with regard to the impacts of transgenic organisms on natural microbial environments.

Direct Effects of GMOs on Soil Microflora

First generation transgenic plants and animals, developed through applications of modern biotechnology, usually contain single gene modifications (deletions, insertions, altered regulation). These simple modifications affect the phenotype of the organism, with the objective of adding commercial value to the transgenic crop or animal. The added value may benefit the biotechnology industry, the farmer or the consumer, or some combination of these. Depending on the nature of the modification, an outward change in the normal array of host–microbe associations may or may not occur. Even under the most simplified of conditions, some change is inevitable. For example, transgenic corn cultivar NK4640Bt expressing the *Bt* toxin gene *cryIAb* exudes some of the toxin protein from the root into the surrounding rhizosphere and soil, along with other proteins normally present in root exudates (Saxena et al., 1999).

Another obvious route of transgene product exposure in soil is via incorporation of plant material into the soil either during the growing season or post-harvest. Transgenic cotton var. Coker line 81 (*cryIAb*) and line 249 (*cryIAc*) release measurable quantities of the truncated *Bt* toxin during decomposition when incorporated into soil (Palm et al., 1994). Cotton line 81 released 10- to 20-fold more toxin than line 249, commensurate with the level of expression of the *Bt* toxin in the plant tissues.

These routes of transgene product exposure are novel and will likely elicit a response from the rhizosphere and soil microbial community. For proteolytic microbes in the rhizosphere, novel proteins or peptides represent an additional source of nutrients (peptides, amino acids, carbon and nitrogen) and they will respond, through the action of extracellular proteases, by degrading the novel protein and assimilating the components. This is the underlying cause, along with physical/chemical processes of protein degradation, of the exponential decay of *Bt* toxin in soil (Tapp and Stotzky, 1998). Plant root and soil microbial proteases can degrade active *Bt* toxin to inactive peptides within days in artificial soil-free media (Koskella and Stotzky, 1997). In real soils, the protoxin can bind to clay and humus materials and this delays proteolytic degradation, in some cases for months (Saxena et al., 1999). During this phase of novel protein persistence, there may be effects on the range of interacting species from different trophic levels in soils; from bacteria and viruses to protozoans, metazoans and insects.

An important consideration from the ecological perspective is whether release of a single novel protein into the soil microbial community is significant in terms of the effect on soil function. Does the incorporation of novel proteins and peptides into soil have a significant effect on the community structure or biodiversity of the associated microflora and, if it does, is there any reason to be concerned about such a change? Some preliminary studies have addressed this issue (Tomlin, 1994; Donegan et al., 1995; Doyle et al., 1995; Donegan et al., 1997; Heuer and Smalla, 1999; Lottmann et al., 1999; Siciliano and Germida, 1999). Other examples of potential direct effects of transgenic organisms on ecosystem processes have been reviewed recently (Kirk, 2000).

While initial studies of rhizosphere microbial diversity using phenotypic measures indicated differences between the microflora of transgenic versus wild-type canola cultivars (Donegan et al., 1995; Siciliano and Germida, 1999), subsequent studies have shown that these differences can reflect soil microbial community patchiness or heterogeneity within the study area (Germida et al., pers. comm.).

In other words, variation in community structure between patches in different plots can overshadow variation due to the presence/absence of transgene products in the rhizosphere. Similar findings were reported by researchers in Braunschweig, Germany examining the microbial flora associated with transgenic T4-lysozyme-producing potato (*Solanum tuberosum*) grown under greenhouse and field conditions (Heuer and Smalla, 1999; Lottmann et al., 1999). In these

studies, small shifts in species abundance were detected, but the observed effects were minor relative to the natural variability observed in several field samplings.

The potential for GMOs to affect critical soil biogeochemical cycles has been raised. Such an effect would require that steps in specific biogeochemical cycles carried out by microorganisms be inhibited or enhanced, presumably as a result of toxicity to the species involved or a shift in community structure. Arguing against such potential effects is the observed redundancy of functions in microorganisms that are involved in many, if not all, biogeochemical cycles. For an illustration of the redundancy of function in a typical soil biogeochemical function, consider a single step in the nitrogen cycle, the chemoautotrophic oxidation of ammonia to nitrate (Aakra et al., 2000). Despite sampling problems in this study (problems that are associated with most analyses of soil microbial diversity), ammonia-oxidizing bacteria were found to be represented by a complex set of taxonomic clusters ("species") within the *Nitrosospira* genus. Unless all of these ammonia-oxidizing species were simultaneously inhibited by the introduction of a GM crop, the function of ammonia oxidation in the soil nitrogen cycle is unlikely to be affected. Of course, it is not inconceivable that the explicit purpose of a biotechnology product (a crop, a microbial inoculant, or an engineered biochemical process) would be to change a step in a biogeochemical cycle. To build on the example above, it may be beneficial to the agronomist, for example, to enhance the oxidation of ammonia to nitrate in the rhizosphere of the crop plant, in order to enhance plant nutrient uptake. In this case, where the biotechnology is in fact designed to modify biogeochemical cycles, risk assessments should be designed to weigh the ecological effects of such a modification. Test systems have been developed to measure such effects (Stotzky, 1993; Jepson et al., 1994).

Lateral Gene Transfer

Gene transfer between closely related and very distantly related microorganisms is an integral part of species evolution in microbial communities. This process can be measured directly (Hoffman et al., 1994; Nakatsu et al., 1995; Dröge et al., 1998; Gebhard and Smalla, 1999; Sengeløv et al., 2000) and it can be inferred from comparative gene or genome analyses (Sundin and Bender, 1996; de Souza et al., 1998; Di Gioia et al., 1998; Ochman et al., 2000; Reid et al., 2000).

Comparative genomics has enabled us to estimate the impact of lateral gene transfer on microbial evolution. Different microbial species vary in the degree to which their genomes are composed of laterally transferred elements. For instance, in the common digestive bacterium *Escherichia coli* K12, approximately 16% of the genome (or about 700 genes) can be attributed to lateral gene transfer within "recent" evolutionary history (Ochman et al., 2000). To give some perspective on what is meant by "recent", the same methods of comparison yield an estimate that

approximately 16,000 nucleotide base pairs have been successfully introduced into the *E. coli* genome per million years. This mobile fraction of the genome is composed of genes or other elements either having the hallmarks of lateral gene transfer function (phage-, plasmid- and transposon-related genes) or having DNA of atypical nucleotide sequence composition or patterns of codon usage that distinguishes it from the rest of the genome. Other species of bacteria that occupy less variable and environmentally challenging places may have much less laterally acquired or foreign DNA. For instance, many parasites (*Mycoplasma genitalium*, *Rickettsia prowazekii*, *Borrelia burgdorferi*) have less than 1% laterally transferred DNA. On the other hand, microorganisms inhabiting highly variable habitats that are subject to periodic disturbance, such as soils, sediments or water, are likely to contain greater proportions of laterally transferred genes. Examples include the cyanobacterium *Synechocystis* PCC6803 and *Pseudomonas putida* (Ochman et al., 2000, http://www.qiagen.com/sequencing/psputida.html, Oct. 2000).

The contribution of lateral gene transfer to microbial genome evolution can be appreciated by looking at the emergence of beneficial bacteria, such as those that remediate toxic organic pollutants in the environment, and pathogenic bacteria, such as those that cause disease in humans. Recent studies of the emergence of pathogenic *E. coli* have shown that lateral gene transfer of virulence determinants has occurred repeatedly during the divergence of different pathogenic strains (Reid et al., 2000). For example, the important food- and water-borne pathogen *E. coli* O157:H7 has acquired numerous pathogenicity determinants over the course of its 4.5-million-year evolution as an animal pathogen (Hacker and Kaper, 2000; Morschhauser et al., 2000; Reid et al., 2000). Since the genome of this pathogen has recently been sequenced, a good perspective on the contribution of gene transfer to the emergence of *E.coli* O157:H7 as a pathogen will be forthcoming (Perna et al., 2001). Similar hallmarks of horizontal gene transfer mark the *Salmonella typhimurium* genome (Baumler, 1997).

The examples listed above of gene transfer between different species or genera are very likely gross underestimates of the degree to which lateral gene transfer determines the structure of microbial genomes. This is because the comparative methods used in these studies are less effective at inferring transfer of genes between more closely related species where gene structure is more similar. In addition, lateral gene transfer of this type occurs far more frequently than transfer between distantly related species, thus compounding the underestimate. Therefore, precautions implicit in many regulatory schemes (see Chapter 3) that pertain to microorganisms and that call for information on the capacity of the microorganism to undergo transformation, transduction and conjugation, should take into account the fact that probably all microorganisms take part in these processes of gene exchange, and that in most environments there will be no possibility, and likely no need, to prevent these processes.

In the face of extensive mixing of genes by lateral gene transfer and rapid generation times by simple binary fission, how are bacterial species identities maintained? As indicated in the introductory paragraphs of this section, we base our definition of a microbial species on a suite of structural, physiological and biochemical features and/or arbitrarily defined differences in gene sequences. Lateral gene transfer will erode these differences. On the other hand, the diversity of microbial niches that exist in most natural habitats ensure that unique taxa are selected that are specially adapted to their niche. These taxa will carry a largely invariant set of essential genes, often termed "housekeeping" genes, that are rarely subject to lateral gene transfer or are not selectively advantageous if they are transferred to a new host.

Under this microbial evolutionary paradigm, what is the significance of introducing a foreign gene or set of genes from a crop, animal or other biotechnology product? We cannot know exactly because we cannot know the entirety of interactions and effects that may arise in microbial communities that remain largely uncharacterized. We can discuss some potential risks, from the perspective of our very limited understanding of microbial community structure and function.

Transfer of Antibiotic Resistance Genes

The biotechnology industry has indicated it is no longer developing crops carrying antibiotic resistance markers for commercialization, and a similar trend is likely to occur in the development of transgenic animals for environmental release. There are alternatives to antibiotic selection in the development of transgenic crops, as there are ways to eliminate these genes from the final construct prior to commercialization (Carrer and Maliga, 1995; Iamtham and Day, 2000). These methods were first developed in bacterial systems and have long been available for microbial GMOs (Sanchez-Romero et al., 1998; van Elsas et al., 1998). Many reports and commissions have recommended that the use of genes conferring resistance to human or animal therapeutic antibiotics be avoided in all circumstances where lateral transfer of these genes may occur. Therefore, this potential risk is considered here only in the context of some existing crops, and as background information for understanding the risks of transfer of other genes.

Antibiotic resistance genes are believed to have been derived in many cases from the very microorganisms that produce antibiotics in soil or aquatic habitats. They are found in bacteria isolated from natural environments with no prior, deliberate exposure to antibiotics (Smalla et al., 1993; Dröge et al., 1998) and they can be found in bacteria isolated prior to the era of human discovery and commercialization of antibiotics. The widespread use of antibiotics since the 1940s has resulted in the selection of antibiotic-resistant strains. The latter have acquired resistance genes either by spontaneous mutation of DNA within the strain or by horizontal transfer from another organism (Walsh, 2000). Natural gene mobility contributes an important dimension to the

rise of antibiotic resistance in human, animal and plant commensal and pathogenic microorganisms (Health Canada, 1993; Sundin and Bender, 1996; Wireman et al., 1997; Heuer et al, 2000; Lawrence, 2000; Walsh 2000). This history of genetic change within organisms that represents a significant threat to our health and the health of our agricultural systems should be taken as an illustration of the importance of selection.

The Importance of Evaluating Selection

Selection will play a crucial role in determining whether or not a particular gene used for modifications to microorganisms, crops or domestic animals poses a threat to other organisms as a consequence of lateral gene transfer. It is impossible to generalize about the magnitude of this risk, as each gene construct will have a different potential for transfer and, more importantly, for selection in the recipient organism. For instance, the rate of acquisition of foreign DNA of 16 kb (or about 16 genes) per million years for *E. coli* discussed above is for "successful" integration of foreign genes, under natural selection pressures. Artificial selection accelerates the rate of successful acquisition of foreign genes by orders of magnitude, as determined for both antibiotic resistance genes and pollutant biodegradation genes (Sundin and Bender, 1996; Di Gioia et al., 1998; de Souza et al., 1998). Most bacterial genomes maintain less than 10 Mbp of DNA, and as genes are acquired through lateral transfer, they are also subject to mutation, recombination and deletion. As a result, the genome size remains more or less within the optimal range for that species in its natural habitat, while the genetic makeup of the organism remains in flux. In other words, microorganisms can be viewed as "sampling gene space" rather than accumulating genes (Ochman et al., 2000). The speed with which adaptive mutations can change microbial population structure has been elegantly demonstrated in laboratory evolution studies conducted over 25,000 generations using *E. coli* (Papadopoulos et al., 1999; Schneider et al., 2000). A large part of the genomic plasticity of this laboratory strain, grown under environmentally stable conditions, has been shown to be due to transposition of insertion sequences (chromosomal mobile genetic elements). This inherent genetic instability therefore contributes substantially to the normal evolutionary change of this species, and by inference all other microbial species. Lateral gene transfer, together with rearrangements and recombination events in recipient organisms, act as driving forces in determining the structures of microbial operons and chromosomes (Lawrence, 2000).

Over the past decade or so, researchers have focused almost all of their efforts on the question of whether or not transgenes and antibiotic resistance markers in plants or animals will transfer to bacteria in the environment. Almost no effort has been expended on the questions of whether or not the genes will be selected in the natural environment and whether or not these genes will pose a risk (Syvanen, 1999). To date, there is no evidence that lateral gene transfer

from transgenic crops to the natural microflora of soil has had a significant effect on soil quality or functional ecology. It has proven quite difficult to detect transfer, although there is some evidence that it can occur under somewhat artificial circumstances (Hoffman et al., 1994; de Vries and Wackernagel, 1998; Gebhard and Smalla, 1999). The difficulty here is not so much in detecting a rare event, as in predicting *a priori* the likely routes of gene transfer, which might be quite complicated. These routes might include uptake of genes from plant residues (Sengeløv et al., 2000) and following animal tissue decay; via ruminant and non-ruminant gut microorganisms and feces (Schubbert et al., 1997, 1998); through plant pollen, root cap cell or root hair cell release; and via a myriad of intermediate vectors including pollinators or their associated microflora (Poppy, 1998; Ramsay et al., 1999); Kaatz study on gut contents of bees (Univ. of Jena); via epiphytic bacteria and insects, or via "rafting" on dispersed particles of plant or soil materials. More importantly, to date there has been no evidence for natural selection acting on any new hosts of genes transferred from transgenic plants or animals. This does not mean that selection cannot operate on these genes. For instance, it is known that mercuric ion released from dental amalgam is at a sufficient concentration in the gut to select for mercury-resistance and genetically linked antibiotic resistance genes in the natural gut bacteria of primates (Wireman et al., 1997). This finding illustrates the subtle nature of selection processes that may come into play.

Where the potential risks of a transgene warrant the cost of the research, case-by-case evaluations of the potential for gene transfer and selection should be done. These studies should place research emphasis on likely means of selection of the transgenes following transfer and how this selection could affect target or non-target microbial communities and ecological processes. Without selection, lateral gene transfer is of little consequence.

RECOMMENDATIONS

6.1 To the extent that the existing regulations, such as those under the Canadian Environmental Protection Agency and the Canadian Food Inspection Agency Acts (Chapter 3), call for ecological information on the fate and effects of transgenic biotechnology products on ecosystems, the Panel recommends that this information should be generated and should be available for peer review.

6.2 If environmental risks are a concern for a particular biotechnology product, especially with respect to persistence of the organism or a product of the organism, persistent effects on biogeochemical cycles, or harmful effects resulting from horizontal gene transfer and selection, then the Panel recommends that exhaustive and long-term testing for these ecological effects be carried out.

6.3 The Panel recommends that, in evaluating environmental risks, scientific emphasis should be placed on the potential effects of selection operating on an introduced organism or on genes transferred to natural recipients from that organism.

6.4 The Panel recommends that a detailed analysis be undertaken of the expertise needed in Canada to evaluate environmental effects of new biotechnology products and, if the appropriate expertise is found to be lacking, resources be allocated to improving this situation.

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PART 2: GM PLANTS

Environmental Risks

One of the most commonly perceived risks associated with GM crops concerns the possibility that transgenes will escape from the confines of agriculture with serious environmental consequences. Indeed, the US NRC report (1989, p. 3) considered that “the potential for enhanced weediness is the major environmental risk perceived for introductions of GM plants.” Two questions that relate to this issue are commonly asked. Will crops that have been GM become invasive? Could the transfer of genes from transgenic crops to their wild relatives through natural hybridization result in the origin of more aggressive weedy types? Under both scenarios, so called “superweeds” are predicted as the unintended results of biotechnology, resulting in the genesis of novel biological invasions. Such invaders would not only reduce crop yields but could also cause serious disruptions in the functioning of natural ecosystems and losses in biodiversity. Because of the potential ecological hazards posed by transgene escape, a considerable literature on this topic has developed during the past decade (reviewed in Tiedje et al., 1989; Crawley, 1990; Ellstrand and Hoffman, 1990; Raybould and Gray, 1994; Snow and Palma, 1997; Warwick and Small, 1998). Recent theoretical and empirical work concerning the potential escape of transgenes from the crop environment enables some assessment of the likely environmental risks posed by GM crops. In this section, we review this work and also consider other ways in which GM crops may have undesirable environmental consequences.

Could GM Plants Become Invasive?

The likelihood that GM crop plants will become invasive and constitute serious weed problems is often considered fairly remote. This is because most of today’s major crop species (e.g. corn, rice, wheat, beans) have been subjected to intense artificial selection over long periods of time for traits with low survival value under most natural conditions. Traits such as non-shattering of grain in cereals, weakly developed chemical defences, lack of seed dormancy, and high fertilizer requirements restrict the ability of most domesticated species to thrive outside the crop environment. Indeed, although crops are grown over vast areas of the globe today there are relatively few cases in which they persist without deliberate cultivation for more than a few seasons. Such volunteer plants are usually confined to agroecosystems and rarely if ever invade undisturbed natural plant communities. Domesticated crop plants are not represented among the world’s serious plant invaders. This is because persistence in wild communities results from the combined effects of many genes working in cooperation to produce a functioning phenotype adapted to local ecological conditions. Therefore, in most cases insertion of specific transgenes into a crop species already possessing a syndrome of domesticated traits is unlikely to alter its

ecology so that it becomes converted into an aggressive invading species. Such targeted genetic modifications are unlikely to nullify many generations of artificial selection involving countless genetic loci.

This argument clearly depends on the extent to which a particular domesticated species has been subjected to artificial selection. Many cultivated species, especially those involved in horticulture, forestry and rangeland agriculture, have only recently been brought under cultivation and consequently have been subjected to relatively little genetic alteration through conventional breeding. In this case, the degree of domestication may be quite minor and cultivated genotypes may resemble their wild ancestors in many respects. In these cases, cultivars are more likely to persist outside of cultivation, and under certain circumstances could become invasive (see below). GM species with a short domestication history are more likely to pose environmental problems than our major crop plants. However, invasiveness will occur only if the genetic modifications increase the survival and reproduction of cultivars in natural ecosystems. Little work in this area has been conducted. In the future, as the range of target organisms for genetic modification widens, it may not be safe to assume that all cultivated species have been genetically crippled through intense artificial selection. Indeed, recent experience in Canada with herbicide-tolerant canola (oil seed rape), discussed next, provides a warning that some crop plants have the potential to become serious weeds of agriculture.

Canola is a relatively recent plant domesticate compared with many of our major cereals (e.g. corn, wheat, rice). Unfortunately, two wild traits that persist in many canola cultivars are weak seed dormancy and a degree of seed shattering. As a result of these traits, large numbers of seeds enter the soil after cropping and can persist in the seed bank to emerge in subsequent seasons as volunteer plants (Pekrun et al., 1998; Derksen and Watson, 1999; Downey, 1999). Traditionally, volunteer crop plants occur at relatively low densities and are eliminated from crops by selective herbicides. However, this management tool is complicated if volunteers are herbicide resistant. Unfortunately, herbicide-resistant volunteer canola plants are beginning to develop into a major weed problem in some parts of the Prairie Provinces of Canada. Indeed, some weed scientists predict that volunteer canola could become one of Canada's most serious weed problems because of the large areas of the Prairie Provinces that are devoted to this crop. Of particular concern is the occurrence of gene exchange via pollen among canola cultivars resistant to *different* herbicides. This can occur through crosses between volunteer plants and the crop, or between different volunteer plants. Three classes of herbicide-resistant canola (resistant to glyphosate, glufosinate and imidazolinone) are currently grown in western Canada. Recent evidence indicates that crosses among these cultivars have resulted in the unintentional origin of plants with multiple resistance to two, and in some cases three, classes of herbicide (Derksen and Watson, 1999; Downey, 1999; Topinka et al., 1999). Such "gene stacking" represents a serious

development because, to control multiple herbicide-resistant volunteer canola plants, farmers are forced to use older herbicides, some of which are less environmentally benign than newer products. This example involving the origin of multiple herbicide-resistant canola serves to illustrate the dynamic nature of weed evolution within managed agroecosystems. It also demonstrates that crops plants are not immune from becoming weeds of agriculture under the appropriate selection regimes.

Because of the large areas devoted to herbicide-resistant canola in the Prairie Provinces, it is not surprising that opportunities for the genetic mixing of different varieties occur. Despite the best efforts of growers, seeds may often be transported accidentally between fields containing different herbicide-tolerant canola varieties by farm machinery, or simply be blown from trucks transporting seeds to and from fields (Gray and Raybould, 1998). Indeed, it has been argued that seed spillage, a form of gene dispersal, may be a much more common mechanism resulting in hybridization between varieties than is likely by long-distance pollen flow by animal pollinators (McHughen, 2000, p. 166). Regardless of the mechanisms giving rise to multiple herbicide-tolerant canola varieties, this example illustrates the problems in trying to predict the likelihood of gene flow from small-scale test plots involving relatively small numbers of plants. In addition, it emphasizes the inherent difficulties in the containment of genetic material in the context of normal farming practices in which literally millions of small seeds are produced and harvested over large areas of the landscape. Industry argues that as long as "good farming practices" are followed, these problems should not occur. This perspective may be unduly naïve. Environmental assessments associated with the release of GM crops should take account of the fact that in the real world human error and expediency may often compromise guidelines for the growing of such crops.

Gene Flow Between GM Crops and Wild Plants

In contrast to many animal species, reproduction in plants can be quite promiscuous. Individuals can mate simultaneously with many partners including themselves and, in addition, hybridization with related taxa commonly occurs. Mating complexity is promoted by a fundamental feature of plant reproduction — plants are immobile and therefore require vectors (e.g. mostly animals or wind) to transport their gametes from plant to plant to ensure cross-fertilization. The process of pollen dispersal is inherently imprecise and only a small fraction of the large number of male gametes produced by a plant (usually < 1%) reach conspecific stigmas resulting in successful pollination. The majority of gametes are lost to the vagaries of the pollination process while a small fraction is dispersed to stigmas of other plant species. If the pollen donor and recipient are related, an opportunity is provided for inter-specific hybridization. Most inter-specific hybrids are genetically sterile or possess maladapted trait combinations and are

soon eliminated by natural selection. Others persist through clonal propagation, while a small minority can become successful new forms because they possess novel phenotypes. Historically, inter-specific hybridization has played an important role in the evolution of flowering plants, with a significant proportion (estimates range from 30% – 50%) of all species arising in this manner.

A major environmental concern associated with agricultural biotechnology is that gene flow from GM crops to related weeds will result in the formation of novel weed phenotypes that have the potential to become highly invasive. Considerable effort in recent years has been directed toward understanding how likely this process is to occur for particular crops, and how to mitigate any negative environmental consequences that might result from such accidental gene transfer (Ellstrand and Hoffman, 1990; Jorgensen and Andersen, 1994; Kareiva et al., 1994; Raybould and Gray, 1994; Snow and Palma, 1997; Lavigne et al., 1998; Rieseberg et al., 1999). Indeed, one of the primary motivations for the use of maternally inherited genetic constructs has been that these technologies will reduce transgenic escape routes through pollen (Daniell et al., 1998; Gray and Raybould, 1998).

It is worth recognizing at the outset that gene flow between crops and weeds has been known for over a century and is not a unique characteristic of the technique of genetic modification per se. Inter-specific or inter-racial hybrids between crops and weeds are commonplace and have been well studied by weed scientists (e.g. carrots, oats, rice, oilseed rape, sorghum, sugarbeet, sunflower; see Table 2.2 in the US NRC report (2000) and similar tables in Snow and Palma, 1997; Rieseberg et al., 1999). Indeed, this phenomenon has resulted in the evolution of a special class of agricultural weeds known as crop mimics that resemble crops in appearance and/or behaviour and thereby evade detection (Barrett 1983, 1988).

The experimental study of pollen dispersal and gene flow in plants generally indicates that the distribution of pollen dispersal distances is highly leptokurtic (most pollen is dispersed short distances with a steadily declining fraction involved with long-distance dispersal). For example, most pollen in herbaceous plants is dispersed within two to three metres of source plants, with a small fraction being transported up to one kilometre or more (Levin and Kerster, 1974; Lavigne et al., 1998). The two most important determinants of pollen dispersal are the mating system of the plant and dispersion of pollen by wind or animals. In general, predominantly selfing species produce far less pollen than outcrossers, and little of this finds its way to other plants. In contrast, outcrossing species produce significantly more pollen and maximum dispersal distances can be considerable, especially in wind-pollinated species. There is no *a priori* reason why these general principles of pollen dispersal should be different for GM crops; so transgenic pollen should not behave in a manner different from the pollen of non-GM plants. However, comparative studies on this issue need to be conducted to confirm this assumption.

Crops can be roughly divided into three groups with respect to the likely incidence of the natural transfer of genes. 1) No possibility — where wild relatives are absent from the region where the crop is grown (e.g. GM maize, soybean, tomato in Canada). 2) Low possibility — GM crops that are either predominantly autogamous (many cereals) or propagated largely by asexual reproduction and flower rather infrequently (sweet potato, sugar cane). 3) Moderate to high possibility — where the crop is an outbreeder and is being grown in an area where cross-compatible wild relatives occur (e.g canola in many parts of Europe and North America; rice in South East Asia). Current guidelines for the field testing of GM crops recognize these distinctions and recommendations for the size and isolation of test plots reflect the likelihood of gene exchange with wild relatives. A major issue here concerns the issue of scale. Opportunities for gene transfer will be considerably greater for large-scale commercial plantings of GM crops than for small test plots. Hence, generalizations about pollen dispersal distances of commercial planting based on experimental studies of small plots should be treated with some caution.

How is hybridization between cultivated and wild plants studied, and is there evidence for the natural transfer of transgenes from GM crops to weeds? The frequency of hybridization events between crops and weeds has usually been detected by simply observing the occurrence of putative hybrids in close proximity to agricultural fields. The presence of plants with “intermediate phenotypes”, or character combinations predicted from hybridization, signal the occurrence of gene transfer. However, there are two reasons why this approach may greatly underestimate the true frequency of gene transfer. First, many products of hybridization are selected against during the establishment phase and hence do not give rise to viable offspring (see below). Second, hybrids can go undetected because of similarities in phenotype to parental forms. This is especially likely where backcrossing and advanced generation crosses result in hybrid swarms composed of plants spanning the entire spectrum of phenotypic variation encompassed by crop and weed. To avoid these difficulties in estimating the true frequency of gene transfer, researchers have recently used simply inherited genetic markers diagnostic for the parental forms to detect hybridization between crops and weeds (e.g. Luby and McNicol, 1995; Whitton et al., 1997, Wilkinson et al., 2000). Assays of seed families collected from individual plants enable the quantitative analysis of gene transfer.

While there is considerable evidence for crop–weed hybridization, only a few cases have been reported involving experimental trials of GM crops. To our knowledge, there are no known cases involving the escape of a transgene into weed populations from commercial scale plantings. To date, most work has involved the insect-pollinated outbreeder, *Brassica napus* (oilseed rape or canola), which can hybridize with several congeneric species (*B. rapa*, *B. oleracea*) as well as the related wild radish (*Raphanus raphanistrum*). It has been suggested that this species can potentially hybridize with up to nine related taxa (Stewart et al., 1997). Since several of these are

also cross-compatible with other wild *Brassica* species, the pool of species that transgenes could potentially infiltrate is quite large. Chèvre et al. (1997) produced *F1* inter-specific hybrids between oilseed rape and radish, and after four generations in field plots, herbicide-resistant plants with a similar morphology and chromosome number to the weed were established. The authors concluded that under normal agricultural conditions this process is likely to occur only rarely. Wilkinson et al. (2000) used remote sensing to identify areas of sympatry between non-GM oilseed rape and wild *B. rapa* over an extensive area (15,000km^2) of South England. Flow cytometry and molecular markers were used to screen for hybrids. Only one naturally occurring hybrid was found. This was a much lower rate of hybridization than anticipated based on earlier predictions from hybridization rates in adjacent populations of the two species and presumed areas of sympatry (Scott and Wilkinson, 1998).

While the available data are sparse and limited to experimental plots of a single GM crop (oilseed rape) it does indicate that transgenes, not unexpectedly, can be transferred to wild plant species, albeit at a low frequency. Other crop–weed systems in which hybridization occurs more frequently (e.g. rice, Langevin et al., 1990) could pose greater risks. Where crops and interfertile wild plants co-exist in the same area, it is probably safest to assume that some degree of gene transfer will occur over time. It is important to recognize, however, that the process of gene flow from GM crops to weeds by itself does not pose an environmental risk. It is the potential consequences of such an event that is the cause for concern. The ecological outcome of hybridization will depend entirely on whether wild plants with newly acquired transgenes have sufficiently enhanced fitness to cause their numbers to increase in frequency. We address this issue below.

Finally, our focus in this section has been on the transfer of transgenes from GM crops to wild plants. As discussed above for canola, another potential escape route involves the transfer of transgenes to other crops of the same species that are not GM. Where GM crops are grown in the same region as non-GM cultivars, opportunities for cross-pollination exist. Indeed, the likelihood of this process occurring is likely to be higher than for most crop–weed transfers because of the very large population sizes involved in crop plantings and the complete absence of breeding barriers that are likely between conspecific cultivars. Recent reports from various European countries of the contamination of canola originating from Canada with small quantities of GM DNA seem likely to have arisen in this manner. Both GM and non-GM canola are grown over extensive areas of western Canada, facilitating insect-mediated cross-pollination between cultivars. While such cross-contamination is unlikely to pose environmental hazards to wild plant and animal communities, it does raise economic and political problems because of concerns in Europe over the food safety of GM crops discussed elsewhere in this report. In addition, the contamination of non-GM crops with transgenes represents a serious problem for low-input

farming (organic agriculture) and may require much larger isolation distances than have been used to now to ensure the purity of non-GM produce (Moyes and Dale, 1999).

Spread of Transgenes in Wild Plants

Predicting the fate of transgenes in wild plant populations is considerably more difficult than determining whether gene flow between crops and weeds is likely to occur. This is because diverse ecological and evolutionary processes will govern the survival and spread of transgenes once they are incorporated into the genetic backgrounds of wild plants. Determining the ecological and evolutionary consequences of transgene spread in wild populations is one of the central issues in assessing the environmental impact of GM crops. While analytical tools have been developed by evolutionary biologists to measure the strength and direction of natural selection (reviewed in Endler, 1986), these approaches have yet to be applied to GM traits. Our ability to predict the spread of transgenes into wild plant communities is hampered by a lack of empirical data on the fitness costs and benefits of transgenic traits in non-crop species. Moreover, it is important to stress that such information is meaningful only when obtained from diverse ecological contexts. Because weedy plants, the likely first recipients of transgenes, have the potential to migrate to diverse habitats through natural dispersal, genotypes containing engineered traits have the opportunity to be tested by natural selection in countless environmental settings. While in many situations weedy genotypes are likely to be poorly adapted, it would be foolhardy to suggest that appropriate conditions do not exist in nature for successful spread. Indeed, experience suggests that novel phenotypes often succeed in circumstances not predicted based on simple demographic models that do not incorporate ecological variation.

Once transgenes are transferred to wild gene pools, their subsequent fate will be strongly influenced by population size. Because transgenes will initially be present at low frequency, they may often be lost from populations through stochastic processes such as genetic drift. Weed populations are especially vulnerable to stochastic processes since population sizes are often small and frequent colonizing episodes lead to an erosion of genetic diversity (Barrett, 1992). Repeated reintroduction through gene flow from GM crops may be necessary for establishment in some weed populations that are subject to frequent fluctuations in population size. The mating system of the weed species will be critical for determining how frequent the introgression of transgenes into wild gene pools is likely to be. Many weeds of agricultural land are predominantly selfing, reducing the likelihood of gene transfer. However, Bergelson et al. (1998) and Bergelson and Purrington (2002) reported that some herbicide resistant transgenic lines of the annual selfing weed *Arabidopsis thaliana* were roughly 20 times more likely to outcross than mutant plants of the same species. Therefore it may not be safe to assume that all selfing plants are immune from genetic contamination since even predominant selfers usually exhibit low levels of outcrossing.

The spread of transgenes into wild populations will be governed by the benefits that they confer to their carriers in terms of enhanced survival and reproductive success. This will depend on the types of transgene under consideration and their effects on plant phenotype. The first generation of GM crops largely involved genes conferring resistance to herbicides and to various pests and diseases, but new genes associated with stress tolerance (e.g. salt, drought and temperature tolerance) are also likely to become commercially available in the near future. It is not difficult to imagine that the escape of such genes could have potential influences on the ecology of wild plant communities. However, the potential ecological impacts of other targeted genes in the second generation of GM crops (e.g. vitamin-rich rice and increased floral longevity in ornamental species) are more difficult to assess.

Most engineered genes are likely to be ecologically neutral and some may carry fitness penalties to their carriers. In these cases, they are likely to be lost from populations quite rapidly through genetic drift or natural selection. Alternatively, some transgenes may provide a selective advantage within wild populations but predicting which constructs these are likely to be is not an easy task. To assess the ecological impacts of transgene escape, recent attempts (reviewed below) have been made to measure the fitness of GM varieties by assessing the costs and benefits of various transgenes in comparison with unmodified varieties. It is particularly important to determine whether transgenes persist in wild plant populations in the absence of selection to maintain engineered traits (e.g. continued herbicide sprays or pest and disease outbreaks). Alternatively, such genes may be selected against because of the costs that they can exert on plant fitness. Such costs can be caused by pleiotropy, linkage to deleterious genes, disruption of coding regions during insertion or the physiological costs associated with maintaining engineered traits.

Not surprisingly, the results of comparative studies of GM versus non-GM plants have been mixed. Some investigators have found no significant differences in performance, whereas others have demonstrated both costs and benefits to the possession of GM traits. For example, Snow et al. (1999) found no significant differences between transgenic herbicide resistant and non-transgenic plants of *Brassica napus* x *B. rapa* hybrids in both survival or seed production in growth chamber experiments. They concluded that the costs associated with herbicide resistance in the hybrids were probably negligible. Similar conclusions were also reached by Lavigne et al. (1995) following competition experiments conducted under field conditions between herbicide-resistant and non-resistant lines of white chicory (*Cichorium intybus*). In contrast, Bergelson et al. (1996) demonstrated a strong cost to herbicide resistance in the weed *Arabidopsis thaliana*, with a 34% reduction in seed production of transgenic plants compared to susceptible genotypes sown into field plots. One of the only studies demonstrating increased fitness of transgenic plants involves *Brassica napus* containing *Bt cryIAc*, an insecticidal transgene that confers resistance to various caterpillars (Stewart et al., 1997). Insect attacks causing defoliation of non-transgenic

plants favoured *Bt* plants in plots that were initially cultivated but were allowed to naturalize. This study is particularly significant because it involved fitness comparisons in natural vegetation.

To fully understand the dynamics of transgenic escape, large-scale demographic studies are required in which the complete life histories of populations are monitored over several successive years. Crawley et al. (1993) estimated demographic parameters of transgenic and non-transgenic *Brassica napus* in a variety of habitats and climatic conditions over a three-year period in the UK. Despite considerable variation in performance among sites and treatments, they found no evidence that transgenic lines were more or less likely to persist in disturbed habitats than plants that were not GM [and see Linder and Schmitt (1995) and Hails et al. (1997) for additional studies demonstrating similar results in *B. napus*]. Ecological comparisons of other GM crops and their associated weed complexes are urgently needed to assess the likelihood that transgene escape could result in negative environmental consequences.

While most studies to date have failed to demonstrate any strong ecological advantage to transgenic plants in comparison with conventional varieties, this should not be taken as evidence that the ecological risks associated with transgene escape will be always be minimal. Too few GM species have been examined for any broad generalizations to be made. Indeed, given the complex nature of many ecological interactions it may not be easy to make firm predictions in this area. Most workers that have considered the problem of transgene escape in any depth agree that each GM crop and transgene combination has to be considered separately, taking into account both the life history attributes of GM crop–weed complexes and the ecological context in which they occur.

GM Crops and Biodiversity

One of the least understood issues associated with GM organisms is their potential impact on biodiversity. For GM plants we have already considered the escape of transgenes into wild populations resulting in the origin of potentially aggressive weeds. While these weeds would impact agroecosystems first, causing yield reductions and economic losses, they could also lead to losses in biodiversity if they subsequently invaded natural plant communities. Most agricultural weeds are rather poor colonizers of undisturbed vegetation and seem unlikely candidates to invade plant communities. However, as discussed above, future genetic modification of a broader range of plant species, including trees, shrubs and clonal perennials, could potentially lead to the transfer of transgenes into plants with competitive life histories. These types of plants are more likely to be capable of moving into communities that up to now have resisted invasion.

While biodiversity is commonly thought to signify the number and kinds of species in a community, it is important to also recognize that biodiversity includes an intra-specific component, specifically the genetic diversity *within* species. How might large-scale introduction of

GM crops influence this component of biodiversity? One potential impact involves genetic alterations in wild plant populations associated with changing agricultural practices. For example, the widespread introductions of herbicide-resistant crops (HR crops) will undoubtedly influence the spectrum of weeds occurring in and around arable fields. If herbicide usage increases because of HR crops, we might also expect more cases of the evolution of weeds that are genetically resistant to herbicides (HR weeds). Warwick et al. (1999) review this issue for the Canadian situation and point out that selection of HR weed biotypes is highest when a single class of herbicide is used repeatedly and is highly efficacious. There are now over 200 reported cases worldwide of herbicide-resistant weed biotypes since resistance was first reported in 1968 (reviewed in Heap, 1999); of these, nearly 30 have been reported in Canada (see Table 6 in Warwick et al., 1999).

Another serious biodiversity concern is the contamination of wild gene pools of the world's major crop plants by genetic constructs engineered through biotechnology. Because, as discussed earlier, many crops have wild and weedy relatives with which they are fully interfertile, the potential for gene transfer into crop gene pools is a serious possibility. This is especially worrisome where crops are grown in regions of the world where they have originated and are thus in contact with a range of close relatives. For example, in South East Asia where rice originated, several cross-compatible wild and weedy species of rice inhabit wetland environments in and around rice fields. In contrast, the majority of crops that are grown in Canada were domesticated elsewhere and the number of cross-compatible relatives vulnerable to this form of so-called "genetic pollution" is more restricted than in many other regions of the world. Nevertheless, the weedy relatives of several crops grown in Canada, such as canola, carrot, sunflower and sorghum, occur in agricultural fields, a situation that creates opportunities for transgene escape.

Agriculture has resulted in the large-scale global destruction of natural ecosystems with concomitant losses in biodiversity. For example, it has been estimated that 70% of the land surface in the UK is under some form of agriculture, and in Canada and the US equivalent figures are 11% and 52%, respectively (reviewed in Maguire, 2000). A question that is often asked is whether the introduction of GM crops will exacerbate the problem of biodiversity loss or alternatively whether the impacts will be minimal. In Europe, this concern has received much greater public attention than in other parts of the world, presumably because farming and wildlife have co-existed for a much longer period and this has resulted in the development of a distinctive flora and fauna associated with farmland. In Europe, many species are adapted to the habitats associated with agricultural practices such as hedgerows, ditches, hayfields and meadows. The widespread use of broad-spectrum herbicides associated with herbicide-resistant crops could potentially reduce plant biodiversity with direct and indirect influences on vertebrate and invertebrate species. For example, a recent report by Watkinson et al. (2000) drew attention to the

possibility that the use of GM herbicide-tolerant crops could result in severe reductions in weed populations with subsequent negative effects on seed-eating birds. Agricultural land in North America is also important for wildlife (Best et al., 1995; Boutin et al., 1999) and detailed studies are urgently needed to assess the impact of the large-scale growing of GM crops on the maintenance of biodiversity in agricultural ecosystems. We support the view taken by Maguire (2000) that conserving biodiversity is an essential part of sustainable agriculture that is beneficial from both an economic and ecological perspective. Agroecosystems that are sterile wastelands not only have little aesthetic appeal but are unlikely to be ecologically sustainable over the long term.

Perhaps the least appreciated way in which the biodiversity of natural plant and animal communities could be threatened by biotechnological change is through genetic alterations in the ecological amplitude of domesticated plants. As discussed above, one of the most potent forces resulting in the erosion of biodiversity is the replacement of natural ecosystems by agriculture and forestry. In the future, because of increasing pressures on land for food, it may be possible to engineer crops to grow in environments that up to now have been considered unsuitable or at best marginal for arable cropping systems (e.g. salt marshes, deserts, rainforests, mangrove swamps). The expansion of the range of conditions in which agriculture can be practised because of advances in genetic engineering could potentially lead to the extensive loss of wildlands and their constituent biodiversity.

Regulatory Implications

Predicting the environmental risks associated with GM crops is difficult because of the diverse ecological interactions that can potentially occur in agricultural and natural plant communities. Serious ecological impacts could arise following very rare events that would be hard to predict from data collected in conventional ecological experiments conducted at restricted spatial and temporal scales. The sparse knowledge base available concerning the ecology and genetics of GM crops is a major hurdle for sound risk assessment, with important regulatory implications. We recommend that before GM crops are released they should be subjected to a more thorough ecological risk assessment than has been conducted to date. In particular, more effort should be given to following the intent of the current Canadian Environmental Protection Agency guidelines with respect to potential adverse environmental impacts. Industry submissions often satisfy current guidelines through reliance on literature reviews without collecting their own experimental data on ecological impacts. Moreover, the whole focus of environmental assessment occurs within the context of agroecosystems only, with little effort paid on assessing likely impacts on the biodiversity of natural ecosystems. In future, we suggest a staged approach in which any new GM variety is subjected to a series of experimental comparisons with conventional varieties, including the unmodified variety from which it originated. These comparisons should be

conducted under various sets of conditions (e.g. glasshouse, growth chamber, field plots, disturbed natural habitats) reflecting increasing ecological realism. The basic goal of these experimental comparisons is to determine whether the new GM crop differs from conventional varieties in any life-history attribute that is likely to have fitness implications for survival in the wild.

These experimental comparisons should provide the necessary information to make informed judgments related to regulation on whether a new GM variety, or its transgenes, are likely to pose an environmental threat by resulting in an invasion scenario. Another series of experiments is also required to determine the likelihood of pollen-mediated gene flow to related species. In these experiments, plots of various sizes of the GM crop should be established at different distances from target colonies (also of different size) of cross-compatible relatives. This will allow quantitative assessment of how gene flow interacts with distance. These experiments differ from those currently used to determine isolation distances of crop varieties from one another. This is because in the proposed experiments the target organism is a related species, not the crop. The wild species chosen should include all related species that are known to be cross-compatible with the cultivar and occur in the area in which the GM crop is likely to be grown.

Future Research

To address public concern about the potential environmental impacts of GMOs, a sound body of ecological research on this topic is required. While Canadian ecological research ranks highly by international standards, very little research currently being conducted by leading ecologists and evolutionary biologists in the country concerns GMOs. Moreover, in our opinion the quantity and the quality of research on the potential environmental impacts of GMOs is not sufficient to address many of the pressing questions that concern the environmental impacts of GMOs. The reasons for limited study in this area are complex and involve a variety of factors. These include: 1) limited funding from government agencies and industry for basic research on the ecology of GMOs (see our discussion of this in Chapter 9); 2) a failure by industry to recognize and take seriously potential environmental problems; 3) an early lack of interest by academic ecologists in what was seen as an uninteresting and perhaps even trivial research question; 4) the reluctance of the research community to commit limited research dollars to the kind of long-term ecological monitoring required in this area. For these and other reasons, the research capability required to answer satisfactorily the questions that are repeatedly raised by the environmental community and the general public is at present severely compromised.

The initial types of investigation that should be conducted on the environmental impacts of GMOs should grow out of research associated with regulation (see previous section). However, it is hoped that once routine protocols are in place for these environmental assessments more basic

research will be addressed. It seems more likely that novel insights will be obtained if scientists are not constrained by the regulatory framework and are free to ask novel questions about the ecology and evolution of GMOs. Below are suggestions for future research on the potential ecological impacts of GMOs.

1. **Glasshouse and growth chamber studies**: The experimental material (GM crop and immediate ancestor) should initially be compared under uniform growing conditions in the glasshouse and in growth chambers using standard randomized block designs with sufficient replication. An important philosophy behind these comparisons not evident in the current guidelines is that investigators look beyond the normal agronomic traits associated with productivity and consider traits likely to have ecological significance in the context of potential escape scenarios. These comparisons could include several experimental treatments to simulate environmental variation, for example, various nutrient, light or temperature treatments. Traits measured should involve standard life-history variables, including growth rate, timing of reproductive events, reproductive and vegetative allocation, seed production, dispersal potential and seed dormancy. Of particular significance is the search for any unanticipated pleiotropic effects of transgene insertion on fitness traits.

2. **Field trials**: Field trials at several locations within Canada with contrasting climate and environmental conditions should be conducted. Once again, it is important that not just agronomic traits are compared but that a range of life-history variables related to fitness are assessed. Of particular interest for these comparisons is the detection of Genotype X Environment interactions in which the life-history traits of the GM crop vary depending on location. Evidence of these interactions can provide useful information on the plasticity of traits and how they might respond to novel environments. More ecological realism could be built into these field trials by introducing biotic interactions with competitors, parasites, predators and mutualists (see section on insect interactions).

3. **Wild communities**: There are obviously inherent dangers in introducing GM plants into wild communities because of the possibilities of escape. However, unless these types of experiments are undertaken it will not be possible to provide an answer to the question of whether GM plants could invade natural ecosystems. We suggest that in the future researchers consider how these types of comparison could be conducted with safety using isolated sites, quarantine procedures and restricted access to the general public to prevent inadvertent escape. Seeds of the GM plants could be sown into a range of disturbed and undisturbed plant communities and their demography monitored for as long as colonies persist. This approach was used by Crawley et al. (1993) in their

studies of transgenic *Brassica napus* in habitats throughout the UK. By measuring standard demographic parameters, projection matrices can be used to predict population growth and likely invasiveness. It is particularly important to determine whether the GM crop has any capacity for persistence through seed dormancy and the maintenance of a seed bank. Most crops possess no dormancy (although see earlier discussion of volunteer canola) so this seems unlikely. However, since dormancy can have an environmental component, and this trait is critical for survival in most wild communities, it is important that this feature of the life history is subjected to the closest scrutiny.

We also recommend that researchers consider conducting a parallel series of comparative experiments on selected cross-compatible relatives containing transgenes. Here the idea is to introduce the transgene artificially into the wild relative and then observe how these plants differ from other individuals of the same species without the gene. Once again, to ensure ecological realism these fitness comparisons should be made under field conditions in a range of disturbed and undisturbed wild communities that would be carefully monitored to prevent escape. These comparisons are time consuming and clearly cannot be conducted on all cross-compatible relatives of GM crops. The choice of which species to examine should be based on their distribution relative to the potential range of the GM crop and the likelihood of escape.

RECOMMENDATIONS

6.5 The history of domestication, and particularly the time period and intensity of artificial selection, of GM plants should be taken into account when assessing potential environmental impacts. Species with a short history of domestication should receive particularly close scrutiny because they are more likely to pose environmental risks.

6.6 Environmental assessments of GM plants and their particular genetic constructs should pay particular attention to reproductive biology, including consideration of mating systems, pollen flow distances, fecundity, seed dispersal and dormancy mechanisms. Information on these life-history traits should be obtained from specific experiments on the particular GM cultivar to be assessed, not solely from literature reports for the species in general.

6.7 Environmental assessments of GM plants should not be restricted to their impacts on agroecosystems but should include an explicit consideration of their potential impacts on natural and disturbed ecosystems in the areas in which they are to be grown.

6.8 Research data from experiments conducted by industry on the potential environmental impacts of GM plants used in Canadian Environmental Protection Agency assessments should be made available for public scrutiny.

6.9 The Panel recommends that a federally funded multidisciplinary research initiative be undertaken on the environmental impacts of GM plants. Funds should be made available to scientists from all sectors (industry, government and university) with grant proposals subject to rigorous peer review.

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PART 3: ENVIRONMENTAL IMPACT: AN ENTOMOLOGICAL PERSPECTIVE

Many species of transgenic plants currently available have been specifically modified to include genes to increase their resistance to major insect pests. To date, about 40 different genes conferring resistance have been incorporated into plants of economic importance (Schuler et al., 1998). Certain species, such as cotton, have been transformed to produce the delta endotoxins of *Bacillus thuringiensis* (*Bt*), a pathogenic bacterium that has been used as a microbial insecticide for over 50 years (Koziel et al., 1993). Other species, such as potatoes, have been transformed to produce proteinase inhibitors, which may be of plant or animal origin (Ryan, 1990).

Their use offers the potential benefits of increased yields and decreased ecological perturbations caused by the traditional application of chemical insecticides. Furthermore, it has also been argued that increased yields could lead to smaller surfaces being used for agriculture, and that the reclaiming of these lands for natural habitats would favour biodiversity.

However, there are a number of potential ecological/environmental costs that must be evaluated before these crops will be widely accepted for general use, including resistance in the target pest species, as well as the impact on other secondary pest species attacking the host plant, the natural enemies of these herbivores, and other non-target entomo-fauna in the ecosystem.

Resistance in the Targeted Pest Species

The effective lifetime of resistant plant varieties, selected through traditional breeding techniques, is often limited by the appearance of pest strains capable of overcoming these defences. Similarly, the indiscriminate use of chemical insecticides has resulted in the selection of very resistant strains of many major pest species (e.g. Metcalf, 1980), thereby limiting the use of these compounds as effective control measures. There is clear evidence that insects have evolved resistance when *Bt* is sprayed as a biological insecticide (Tabashnik, 1994) so there is no reason to expect anything different with a wide-scale, intensive use of transgenic plants (Gould, 1998). The *Bt* toxin is incorporated directly into the plant through genetic manipulation and the herbivores may be exposed for considerably longer periods during their development than with conventional *Bt* spraying. The appearance of *Bt*-resistant pest populations due to the widespread use of transgenic plants could have at least two undesirable effects: i) *Bt* is the most effective biological insecticide available to organic farmers; the loss of this means of control seriously jeopardizes their livelihood and an expansion of this more ecologically friendly form of agricultural practice, and ii) the possibility of a serious environmental impact if conventional farmers resorted to increased applications of chemical insecticides to control populations when the GM plants no longer offer sufficient levels of protection against pest species. These could be seen as points pertaining to altered pest potential, potential impact on non-target organisms (in this case, organic

growers) and potential impact on biodiversity (items 2.1.3, 2.1.4 and 2.1.5 of the substantial equivalency for GM plants).

It is, therefore, essential that a well-developed resistance management program be implemented whenever the use of transgenic plants is a component of any production system. One approach relates directly to the GM plants. For example, the production of plants expressing very high levels of the toxin/antifeedant (known as the high-dose approach ensuring 100% mortality) would markedly decrease the possibility of resistance evolving. However, while this approach may be theoretically sound, 100% mortality may prove unrealistic under natural conditions. If some pests did survive there would be strong selection for resistance, since one would be eliminating all but the most resistant individuals in the target population. Furthermore, even if the high-dose approach were successful in controlling the pest species, it would be essential to ensure that these high levels of toxin/antifeedant do not result in negative effects at some other level in the system, such as increased toxicity to consumers (see elsewhere in this report) or on other species associated with the agroecosystem (see below).

Another approach is ensuring the existence of refuge populations of the pest species. These populations are not subjected to selection for pesticide resistance, so mating between resistant and susceptible individuals would also slow the process of selection for resistance. However, if developmental asynchrony occurs between susceptible and resistant strains, then assortative mating (mating of like phenotypes: resistant with resistant and susceptible with susceptible) may accelerate the evolution of resistance (see Liu et al., 1999). The practice of planting susceptible host plants in association with GM ones has been employed in the cotton-producing areas of Australia (see Fitt and Wilson, 2000). In this case, the number of hectares planted with non-transformed, unsprayed refuge crops is determined as a function of the land areas planted with GM plants (*Bt*) and non-transformed plants treated with conventional insecticides. However, insect movement is a confounding factor that may modify the effectiveness of resistance management. When highly mobile species are involved, any resistance management strategy must be viewed from a regional rather than local scale, since efforts to manage resistance at one site may be compromised through the immigration of individuals from another area where there has been strong selection for resistance. For example, some of the moth species targeted by the use of the *Bt*-expressing cotton plants in Australia migrate over considerable distances (Fitt, 1989), so the control means deployed at one site may have considerable influence at sites hundreds of kilometres away. An influx of resistant individuals would be a component of the "gene flow" criterion, which is specifically mentioned in the procedure for the determination of substantial equivalence for GM plants.

The idea of insects developing resistance to "insecticidal GM plants" is not a trivial matter, and it is essential that the question of resistance monitoring be addressed immediately to establish

meaningful guidelines for the monitoring of resistance (see Roe et al., 2000). In establishing guidelines one must not only consider the question of migration from other sites, but also movement of the target species between different host plants species within the habitat. For example, if an insect attacks three agricultural crops (corn, beans and potatoes), as well as several uncultivated species, then the introduction of one transgenic crop (e.g. corn) could have a very different impact on potential problems of resistance than if the pest species attacked only corn.

Impact on Other Herbivores Attacking the Same Host Plant

It is very rare that a given plant will be attacked by only one species of herbivore so the possibility that the biology of non-target herbivores may be significantly modified when feeding on transgenic plants cannot be overlooked. In many cases, one would expect similar effects against the different species of herbivore, especially if they have the same feeding strategies (i.e. chewing), although varying susceptibility to *Bt* toxins is known among different species of lepidopteran larvae.

A recent study looked at the performance of the potato aphid, *Macrosiphum euphorbiae*, a secondary pest problem in potato production, on two GM potato lines whose transgene conferred resistance against the Colorado potato beetle (Ashori, 1999). He found that, when compared with control plants, aphids did very poorly on plants expressing the *Bt* toxin. However, on a GM line expressing a proteinase inhibitor, aphids not only survived (as well as on controls) but also had significantly better reproductive success than on control plants. Thus, the use of this GM line potentially could result in higher aphid populations, which would not only increase the risk of reduced host plant performance due to aphid feeding but could also increase the probability of increased spread of plant diseases vectored by aphids.

There are now a number of examples (oral presentations at the 2000 joint meeting of the Entomological Societies of Canada, the United States and Quebec at Montreal) where the use of *Bt* transgenic crops has decreased the number of sprays used against the target pest but has increased problems with secondary pests. In the past, these “minor” pests were controlled by the repeated applications of insecticides against the primary pest but now, in the absence of these sprays, the “minor” pests are able to develop, since they are unaffected by the toxin.

Impact on the Natural Enemies of Herbivores

One of the problems associated with the use of traditional chemical insecticides is the negative impact of these compounds on natural enemies. A decline in natural enemies following spraying frequently allows the subsequent resurgence of the pest species following the initial knockdown effect of the treatment and/or outbreaks of secondary pests (van den Bosch, 1978). The use of GM plants will eliminate the direct negative influence of natural enemies coming into contact with the toxin/inhibitor on the plant surface as it will be contained within the plant tissues. Thus, the deployment of GM plants could result in higher densities of natural enemies than in plots treated with conventional insecticides (Hoy et al., 1998). However, the acquisition of the toxin/inhibitor by all natural enemies is still possible through the ingestion of herbivore tissue. Some studies testing that hypothesis have shown negative effects (e.g. Hilbeck et al., 1998a, b) while others have not (e.g. Hough-Goldstein and Keil, 1991). A number of explanations exist for these apparently conflicting findings with respect to the impact of GM plants on natural enemies.

One obvious source of difference is the type of GM plant being tested, as previously noted with the performance of the potato aphid on different transgenic potatoes. In a laboratory study on *Aphidius nigripes*, the major parasitoid of the potato aphid (Cloutier et al., 1981), mortality from egg to adult was higher in aphids reared on a *Bt* potato line but on an oryzacystatin I (OCI) line was similar to controls (Ashouri, 1999). Furthermore, while their developmental time did not vary, parasitoids from hosts on the OCI line were significantly bigger than controls, thus having a potentially higher reproductive success. However, no clear trends were observed under field conditions (Ashouri, 1999), as the incidence of parasitism was very low during the two years of the study (in part due to the widespread use of traditional insecticides against the Colorado potato beetle in recent years).

There will also be obvious interspecific differences of sensitivity to the toxins, even when the same GM host plant is tested. These may relate to differences in the life histories of the species under consideration: for example, effects may be more pronounced for endoparasitoids, which actually live within the host, than for ectoparasitoids or predators that are external feeders.

The environmental conditions under which the experiments are carried out and the actual assays used could also influence the results obtained. For example, a laboratory study was carried out looking at the possible effects of the ingestion of the cysteine proteinase inhibitor, OCI (from rice and expressed in potato) on the two-spotted stinkbug, *Perillus bioculatus*, a predator of the Colorado potato beetle (Ashouri et al., 1998). In this experiment, the stinkbug females were fed beetle larvae injected with different chronic concentrations of OCI (1–16µg/day). While survivorship was not affected, there were negative dose-related effects on reproduction (longer pre-reproductive period, lower daily fecundity, lower egg mass size and reduced eclosion of eggs). Furthermore, these effects continued for some time after females were provided control

food only, and at the highest doses the effect was non-reversible (Ashouri et al., 1998). However, in another series of experiments the growth of two-spotted stinkbug larvae was studied but in this case the prey (Colorado potato beetle larvae) were actually fed OCI plants rather than being artificially injected with the proteinase inhibitors. No significant differences were observed in survivorship, developmental time or weight gain between predatory larvae attacking hosts fed on OCI or control plants (Bouchard, 1999). Thus, in this system, the quantities of proteinase inhibitors a small predator would ingest via the herbivore had no detrimental effects on the parameters studied.

One must also place the parameters evaluated in any given bioassay within a broader context. For example, as noted above, Ashouri et al. (1998) reported some negative impact on stinkbug females that were fed beetle larvae that had been injected with different chronic concentrations of OCI. However, the authors also carried out feeding assays and found that individuals fed on OCI-injected prey showed a significantly higher incidence of attack than controls. This suggests that the ingestion of the OCI changes gut biochemistry and affects the feedback loop modulating "hunger"(Ashouri et al., 1998). Thus, while having a lower reproductive output, these stinkbugs might have a significantly higher predatory activity. If this occurred under natural conditions, then an increased attack rate by individual predators might compensate for an overall lower population density of natural enemies.

Some natural enemies are omnivores, and thus could ingest the products of transgenes through direct feeding on plant tissues as well as through the ingestion of prey feeding on GM plants. The predatory two-spotted stinkbug may feed directly on the plant, especially early in larval development. Young two-spotted stinkbug nymphs confined on plants without prey did feed on plant juices, with no differences being detected between those feeding on OCI and control plants (Bouchard, 1999). There was clear evidence that the ingestion of OCI did influence digestive protease activity in the predator but, at the concentrations encountered, the animals could compensate (Bouchard, 1999). This is not particularly surprising, since some proteinase inhibitors are also found in non-transformed plants, so some exposure to these compounds will occur under natural conditions.

Other natural enemies may feed on host plant products in specific parts of their life cycle. This is particularly true for adult parasitoids; they use pollen and nectar as food sources which may significantly impact on both their longevity and reproductive success. Given that these species directly ingest plant products, the potentially negative effects of feeding on GM plants must also be evaluated. Does active feeding influence the population dynamics of parasitoids and could this lead to the resurgence of pest populations in a manner similar to that observed when chemical insecticide sprays reduce natural enemy populations?

Furthermore, in order to determine the impact on natural enemies under field conditions one must also consider the number of different host species exploited by a given parasitoid or predator. For example, if a major parasitoid of a cotton pest also exploits many other insect species within the habitat, one must determine the relative importance to the parasitoid of hosts feeding on the GM crop relative to those hosts feeding on non-GM plants. It is clear from the preceding discussion that evaluating the potential impact of GM plants on natural enemies is a complex issue and that a real understanding will only be obtained from well-esigned, ecologically meaningful experiments focused on this issue.

Impact on Other Non-Target Insects in the Habitat

One major plant–nsect interaction relates to pollination, since many plant species depend on insects for successful reproduction. The honey bee, *Apis mellifera*, is a major pollinator of many agronomic crops and,while no detrimental effects have been reported from their exploitation of pollen from current GM plants (Poppy, 1998),additional studies should be conducted. For any given crop there may be a highly diverse guild of pollinator species, but very little work has been carried out investigating the potential impact of GM plants on other pollinators (e.g. bumblebees, solitary bees, syrphids) that use pollen and/or nectar as food. It should be realized that any potential impact will probably not be described by a yes-no response, as possible effects may vary in time and/or space depending on the ecological conditions.

Other plant species are wind pollinated and the direct impact on pollinators of pollen from GM plants using this pollination strategy could be considered negligible. However, the very nature of wind pollination results in pollen being found at different sites throughout the ecosystem. Losey et al. (1999) addressed the potentially negative effects of windborne pollen on non-target species by examining the impact of pollen from *Bt* corn on the survivorship of monarch larvae feeding on milkweed, its normal host plant, which is commonly found near corn fields. This paper attracted considerable public attention but also received considerable criticism concerning the validity of the experimental protocol used (e.g.the high pollen density used). A second study has also reported a negative impact of pollen from *Bt* corn on monarch larvae, this time using pollen loads similar to those found on milkweed plants growing near corn fields (Hansen and Obrycki, 2000). In contrast, a similar study on the black swallowtail showed no detrimental effects when caterpillars ingested ecologically relevant concentrations of pollen from most GM corn plants (Wraight et al., 2000).

Together, the results of these experiments underline two important points: i) one cannot rule out potentially negative impacts of pollen from wind-pollinated GM crops if the pollen is ingested by non-target organisms feeding on other plants in the ecosystem; and ii) there are important species differences in susceptibility. It should also be noted that the susceptibility of a

particular herbivore species to a fixed dose of pollen may be affected by many factors, such as the insect's developmental stage and overall physical condition, and the chemistry of the host plant. For example, would one observe similar levels of mortality of a polyphagous herbivore (one that eats several different species of host plant) when it consumes GM pollen in combination with foliage from two different host plants with very different chemical profiles?

Thus, considerable research will be required to elucidate possible effects of pollen from GM plants, whether they be insect or wind ollinated, if the expression of the transgene is not restricted to those specific parts of the plant (e.g. leaves or roots) attacked by the important pest species. Particular attention is required when wind-pollinated GM plants are grown near habitats of lepidopteran species that are rare or endangered, for if there was a negative impact it could directly contribute to a reduction in biodiversity. For example, in the US the Environmental Protection Agency has called for data examining the potential impact of *Bt* corn pollen on the endangered Karner blue butterfly (Hansen and Obrycki, 2000).

General Conclusions

It is clear from available information that the impact of GM plants on both target and non-target insect species is extremely variable, so rigorous experimentation will have to be carried out on a case-by-case basis to determine potentially negative effects. In the future, it may be possible to draw broader generalizations by considering insects that are closely related phylogenetically or that share similar life-history strategies. For example, are polyphagous species more likely to develop resistance to proteinase inhibitors than monophagous ones, as a result of their normal exposure to a wider variety of naturally occurring enzymes and plant defence compounds? For the moment, however, there are not enough available data to determine if such broader predictions concerning potential outcomes with respect to pests, natural enemies and/or non-target species are possible.

The implementation of rigorous field testing of previously released GM plants, and any coming on line, will help develop the necessary data sets that will permit us to look for possible general trends. It must be borne in mind that data from small field trials may not always provide a realistic picture of the situation that prevails under full commercial production. Therefore, it is essential that there be continued monitoring for those GM crops currently being used on a commercial scale with careful comparisons with conventional agricultural practices. The parameters measured would be similar to those suggested in the protocol for small plots but should be expanded to include monitoring of bird and small mammal populations. Such studies will provide information on the changes, if any, in the biological systems where GM plants are being intensively used.

Other GM Organisms for Insect Control

While insect pathogens (bacteria, viruses, fungi and protozoa) are a component of integrated pest management programs against many insect species, their sales (a reflection of use) pale when compared with those of chemical insecticide (Federici, 2000). In part, this is due to low and variable efficacy, which may be influenced by a wide array of both biotic and abiotic factors. Recombinant DNA technology is seen as one approach that could significantly increase pathogen efficacy, and already field-scale trials of GM pathogens are being carried out in certain countries. However, as with the use of GM plants there are/will be a series of ecological, economic and social questions that must be addressed as these products become available commercially (see Richards et al., 1998).

Biological control agents (parasitoids, predators) are seen as a highly desirable alternative to traditional insecticides although, like pathogens, their efficacy is affected by many environmental factors. Again, although research is ongoing with respect to GM "natural enemy" control agents, there are questions relating to the long-term effects that the presence of these organisms might have on different ecosystems (see Hoy, 2000).

To date, there are no GM microbial pest control agents registered in Canada. However, the information officer at the Pest Management Regulatory Agency (PMRA) indicated to the Panel that registration of these products would follow the same guidelines currently deployed for the registration of conventional microbial pesticides. With respect to natural enemies, permits for importation and release are currently studied by PMRA on a case-by-case basis, with input from the Canadian Food Inspection Agency (CFIA)and Agriculture and Agri-Food Canada. If and when there are genetically altered biological control agents presented for regulatory approval, PMRA indicated there would be a case-by-case review (by PMRA and CFIA) to determine if these organisms were acceptable for use in Canada.

Given the ecological complexity of pest–natural enemy/pathogen interactions, the Expert Panel believes the appropriate governmental agencies should start immediately to consider how the evaluation of these GM organisms will be carried out, specifically addressing questions relating to potential long-term ecological consequences once these organisms are released in nature.

RECOMMENDATIONS

Given the need for transparency in the registration process of GMOs, it is recommended that, when considering applications for registration of new plants with novel traits:

6.10 Companies applying for permission to release a GMO into the environment should be required to provide experimental data (using ecologically meaningful experimental protocols) on all aspects of potential environmental impact as outlined in the current guidelines relating to "substantial equivalence" (e.g. CFIA Step 2 on page 12 of the document Regulatory Directive 95-01 and in Appendix 3 of Regulatory Directive 2000-07).

6.11 An independent committee should evaluate both the experimental protocols and the data sets obtained before approvals are granted.

6.12 Standard guidelines should be drawn up for the long-term monitoring of development of insect resistance when GMOs containing "insecticidal" products are used, with particular attention to pest species known to migrate over significant distances.

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PART 4: POTENTIAL ENVIRONMENTAL RISKS RESULTING FROM INTERACTIONS BETWEEN WILD AND CULTURED FISH

To date, the assessment of environmental risks associated with GM foods in Canada has been restricted to those resulting from transgenic plants and microbes (see Parts 1 and 2). As of November 2000, the Canadian Food Inspection Agency (CFIA) had not received a request for approval of a GM animal for commercial food production. However, when the Canadian government does receive such a request, it will almost certainly be for a GM fish. Given the high probability that CFIA will receive such a request within the next 10 years, the Panel considered it appropriate to examine the potential risks to the environment posed by the commercial production of transgenic fish. To this end, it is important to note the comparatively short history of domestication of farmed fish in Canada, relative to that of crop plants and terrestrial animals. For this reason, coupled with the paucity of environmental and ecological assessments of transgenic fish, the Panel considered it necessary to draw upon research on interactions between wild fish and their non-transgenic, farmed counterparts to provide an empirical basis upon which the potential environmental risks posed by GM fish can be assessed. Also, given its predominance in Canada today and in the foreseeable future, this section focuses primarily on the aquaculture of salmonids, that is, salmon, trout and char.

Salmonid Aquaculture and the Incidence of Escape Events in Canada

By any metric, the Canadian aquaculture industry has experienced impressive growth over the past two decades. By 1998, the Canadian aquaculture industry was producing approximately 92,000 tonnes of product valued at $443 million (DFO, 2000c). By 1999, the farming of salmonid fish alone accounted for 68,000 t (74%) of the total aquaculture tonnage and 92% of the industry's value (DFO, 2000c). Among these fish, Atlantic salmon was by far and away the most highly farmed species, with production estimates of 22,610 t in Atlantic Canada (Whoriskey, 2000) and 30,165 t in British Columbia (Noakes et al., 2000) in 1998. Worldwide, the production of farmed Atlantic salmon has exceeded that of all other organisms reared in aquaculture facilities, experiencing a rate of increase of 22.4% per annum (Naylor et al., 2000).

With reference to Atlantic and Pacific salmon, fish farming involves two main phases, both of which may have consequences for interactions between wild and domesticated species. During the initial freshwater phase, individuals are spawned artificially from broodstock and reared in land-based tanks for usually one to two years. The second phase of the rearing period begins with the transfer of fish to aquatic netpens, or sea cages, where the fish are maintained until they attain a size at which they can be marketed. During both phases of this rearing period, farmed fish are exposed to environmental conditions that differ greatly from those they would normally

experience in the wild. To a greater or lesser degree, then, the unnatural environment results in domestication selection (i.e. differential mortality among farmed individuals), with the greatest survival experienced by those individuals whose physiology, morphology and behaviour provide them with a survival advantage in the farm environments.

Risks to wild fish populations arise from the escape of cultured fish from aquaculture facilities.

Without a remarkable improvement in containment capabilities, the number of escaped domesticated fish interacting with wild fish can be expected to increase significantly if Canada's aquaculture industry maintains its current 15% annual rate of growth (DFO, 2000c). In the only Canadian river (Magaguadavic River, New Brunswick) for which annual data on escaped cultured fish and wild fish exist, the number of cultured fish entering the river between 1992 and 1999 has been two to eight times that of the wild salmon returning to the same river to spawn (Carr et al., 1997; Whoriskey, 2000). On the Pacific coast, the numbers of Atlantic salmon escaping into British Columbia waters averaged 43,863 per annum between 1994 and 1998 (Noakes et al., 2000); an estimated 32,000 to 86,000 farmed Atlantic salmon escaped from netpens between January and September 2000 (Mickleburgh, 2000; Sullivan, 2000). Concomitant with the increased aquaculture production of Atlantic salmon in Pacific waters is evidence of natural spawning by escaped members of this exotic species in British Columbia rivers (Gross, 2000; Volpe et al., 2000).

Genetic and ecological factors will influence the extent to which native populations are affected by interactions between wild and escaped aquaculture fish, whether the latter are transgenic or not. Genetic interactions can result in the exchange of genetic material, or introgression, between wild and cultured forms of the same species, or less frequently between cultured fish of one species and wild fish of another species. Intra- and inter-specific ecological interactions involve those related to predation, competition for food, space and mates, and the transmission of disease and parasites between cultured and wild fish. Regarding the relative importance of ecological versus genetic factors, it is important to note that an absence of gene transfer between wild and cultured fish need not significantly reduce potentially negative population consequences to wild fish. The well-documented negative effects of exotic species introductions to wild ecosystems underscore the point that organisms need not interbreed for negative impacts to population persistence to be realized. This may be particularly important when intrusions by cultured fish are frequent, involve relatively large numbers of cultured fish, and when wild population sizes are near historically low levels.

Genetic Interactions Between Wild and Cultured Fish

The effect of genetic interactions on the viability and persistence of wild fish populations will depend on the degree to which individuals are adapted to their local environment, on the genetic differentiation between wild and cultured individuals, on the probability and magnitude of outbreeding depression (i.e. a fitness reduction in hybrids from matings between individuals from two genetically distinct populations), and on the size of potentially affected wild populations relative to their carrying capacities (Hindar et al., 1991; Hutchings, 1991a).

Local Adaptation in Fish

There is considerable evidence of adaptation by fish to their local environments (see reviews by Hindar et al., 1991; Taylor, 1991; Carvalho, 1993; Conover and Schultz, 1997; Lacroix and Fleming, 1998). This adaptive variation can be evident among fish inhabiting different lakes, rivers, or even tributaries of the same river. Notwithstanding suggestions to the contrary (Peterson, 1999), evidence of outbreeding depression (see below), population differences in resistance to disease, and adaptive variation in growth rate and life history (Table 2) are inconsistent with the hypothesis that the introduction of genes from one population is, in general, likely to increase the fitness of individuals within another population.

Most research on transgenic fish in Canada is directed toward the production of growth-enhanced fish for the aquaculture industry. Thus, of particular relevance to the question of whether genetic interactions between wild and transgenic fish may have deleterious consequences to native populations is the substantive evidence of adaptive, among-population variation in individual growth rate (Table 2).

Genetic Differences Between Wild and Cultured Fish

There is a high probability that non-transgenic cultured fish differ genetically from their wild counterparts (Crozier, 1993; Fleming and Einum, 1997; Clifford et al., 1998). These differences are generated by the very different environments, and corresponding selection pressures, in which wild and cultured fish spend their lives. Selection in the wild generally represents weak stabilizing selection for traits that optimize individual fitness in the natural environment. By contrast, selection in hatcheries and in farms is directional (e.g. selection for faster growth, larger body size, increased aggression), favouring traits, with unknown correlational effects, that optimize marketability, rather than the ability to produce offspring in the wild that they themselves will survive to reproduce successfully. It is improbable that selection in the natural and cultured environments will be similar. Selection on individual growth rate, for

Table 2. Selected examples of evidence for local adaptation in fish

Species	Trait	Reference
American shad	age at maturity	Leggett and Carscadden (1978)
brook trout	egg size	Hutchings (1991b)
	age at maturity	Hutchings (1993)
Atlantic salmon	age at maturity	Hutchings and Jones (1998)
	parasite/disease resistance	Bakke (1991)
	growth rate	Torrissen et al. (1993)
sockeye salmon	breeding time	Hendry et al. (1999)
coho salmon	parasite/disease resistance	Hemmingsen et al. (1986)
sockeye salmon	migratory behaviour	Quinn (1982)
Atlantic cod	resistance to cold waters	Goddard et al. (1999)
	growth rate	Svasand et al. (1996)
	plasticity in growth rate	Purchase (1999)
	plasticity in behaviour	Puvanendran and Brown (1998)
largemouth bass	growth rate	Philipp and Whitt (1991)
mummichog	growth rate	Schultz et al. (1996)
Atlantic silverside	growth rate	Conover and Present (1990)
striped bass	growth rate	Conover et al. (1997)

example, can be particularly intense in cultured environments and the response to selection has been remarkably high (e.g. 8–10% per generation in Atlantic salmon (Gjoen and Bentsen, 1997), and 50% over 10 generations in coho salmon (Hershberger et al., 1990).

By definition, transgenic fish differ genetically from their wild counterparts. Although some of these differences will be manifest by obvious differences in phenotype, such as differences in size at age, others may not. The latter will be particularly important for physiological traits such as cold-water resistance, salinity tolerance, and ability to metabolize plant protein, characters of interest for future biotechnology research in fish. This is an important point when assessing the environmental risks of transgenic fish. Depending on the transgene, some transgenic individuals may be phenotypically, behaviourally or physiologically similar to their wild counterparts, increasing the difficulty of assessing potential risks of cultured fish escapees on wild fish populations.

Hybridization and Outbreeding Depression in Fish

Relative to other animals, fish tend to have relatively high levels of inter-specific hybridization, presumably because of their high propensity for external fertilization (Hubbs, 1955; Chevassus, 1979). Hybridization can be expected to be more frequent between species that have had a comparatively short history of cohabitation (e.g. between native and introduced fish). In the present context, Hindar and Balstad (1994) reported that, from 1980 to 1992, hybridization between Atlantic salmon and brown trout in Norway increased almost four-fold with increased production of farmed Atlantic salmon (escapees typically comprise 20%–40% of the size of wild populations in Norway, reaching as high as 80%; Fleming et al., 2000; Mork, 2000).

Although risks to wild fish resulting from inter-specific hybridization may be comparatively low, the potential consequences of mating between wild and cultured members of the same species merit close attention.

The fitness of offspring resulting from matings between wild and cultured fish of the same species can be reduced, relative to the fitness of pure-bred wild offspring from the same population, possibly because of the breaking up of co-adapted gene combinations found within the wild populations. Evidence of such outbreeding depression for characters such as survival, disease resistance and growth rate has been well documented in fish (Table 3). Within the present context, and based on experiments in the wild, differences in viability in early life and juvenile growth rate between farmed, first-generation hybrid, and wild Atlantic salmon have revealed negative influences of intra-specific hybridization between farmed salmon and wild salmon, a finding consistent with the hypothesis of outbreeding depression (McGinnity et al., 1997; Fleming et al., 2000). And in the only analogous study to date on transgenic fish, Muir and Howard (1999) documented a significant reduction in survival among the progeny of GM medaka relative to those produced by pure non-transgenic crosses.

It is also important to note that the fitness consequences of outbreeding depression may not be realized immediately in first-generation hybrids if these fish retain intact components of parental genomes, thus maintaining the inter-gene, or epistatic, interactions favoured by natural selection; these interactions may not be disrupted until the second generation, or later, after recombination has occurred.

Ecological Interactions Between Wild and Cultured Fish

Interactions Between Wild and Non-Transgenic Cultured Fish

Ecological interactions between wild fish and cultured fish that have escaped from aquaculture facilities can be broadly categorized as those resulting from competition for resources,

Table 3. Evidence of outbreeding depression in fish

Cross	Species	Trait(s)	Reference
wild x wild	pink salmon	survival	Gharrett et al. (1999)
	coho salmon	parasite resistance	Hemmingsen et al. (1986)
	mosquito fish	growth rate	Leberg (1993)
	sockeye salmon	survival	Wood and Foote (1990)
	largemouth bass	survival	Philipp and Whitt (1991)
wild x farmed	Atlantic salmon	survival	Fleming et al. (2000)
non-transgenic	Atlantic salmon	survival	McGinnity et al. (1997)
	Atlantic salmon	growth rate	Fleming et al. (2000)
wild x transgenic	medaka	survival	Muir and Howard (1999)

such as food, space and mates, those resulting from predator–prey interactions, and those resulting from disease and parasites (Hindar et al., 1991; Hutchings, 1991a; Fleming et al., 1996; Gross, 1998; Lacroix and Fleming, 1998; Whoriskey, 2000).

Competition between cultured and wild fish for food and territories can negatively affect the growth and survival of the latter and can presumably occur at any age and size. During spawning, competition can be expected for nest sites and for mates, unless there are significant temporal differences in the timing of reproduction. Escaped cultured fish, if they are comparatively large, may prey upon wild fish of smaller size. Depending on the number and size of escaped fish, absolute increases in fish abundance have been hypothesized to increase the mortality of wild fish indirectly either because of increased attraction to natural predators or because of increased fishing pressure by anglers. Transfer of disease and parasites from cultured to wild fish can also represent a potential threat to the persistence of wild populations (although it would be incorrect to assume that all such pathogens have their origin in cultured fish). Of particular concern in North America are bacterial kidney disease (caused by the bacterium *Reinebacterium salmoninarium*), infectious salmon anemia (a disease that resulted in the government-ordered destruction of two million cultured Atlantic salmon in New Brunswick in the late 1990s), and the parasitic sea lice *Lepeoptherius salmonis* and *Caligus elongatus*.

Although the hypothesized consequences of interactions between wild and cultured salmon are many, the number of empirical evaluations of these are few. Nonetheless, it is known that:

- # escaped farmed Atlantic salmon can spawn successfully in rivers in the North Atlantic and the Northeast Pacific (Webb et al. 1991; Volpe et al. 2000);
- # escaped farmed Atlantic and Pacific salmon have destroyed the egg nests constructed by wild salmon (Gallaugher and Orr 2000);
- # the breeding performance of farmed Atlantic salmon, particularly males, can be inferior to that of wild salmon (Fleming et al. 1996, 2000);
- # the progeny of farmed Atlantic salmon (including hybrids with wild salmon) can experience lower survival in early life than progeny of wild salmon (McGinnity et al. 1997; Fleming et al. 2000); and
- # as juveniles, the progeny of farmed Atlantic salmon can compete successfully with, and potentially competitively displace, the progeny of wild Atlantic salmon (McGinnity et al. 1997; Fleming et al. 2000).

Interactions Between Wild and Transgenic Fish

The pleiotropic consequences effected by insertion of single gene constructs in fish (see Chapter 5) presents a major difficulty in reliably assessing the environmental risks posed by transgenic fish. For example, growth hormone constructs in salmonids have been shown to influence smoltification (Saunders et al., 1998), swimming ability (Farrell et al., 1997), gill irrigation (Devlin et al., 1995a,b), feeding rates (Abrahams and Sutterlin, 1999; Devlin et al., 1999), risk-avoidance behaviour (Abrahams and Sutterlin, 1999), disease resistance (Devlin, 2000), muscle structure and enzyme production (Hill et al., 2000), cranial morphology (Devlin et al., 1995a, b), body morphometry (Ostenfield et al., 1998), pituitary gland structure (Mori and Devlin, 1999), life span (Devlin et al., 1995a, b), and larval developmental rate (Devlin et al., 1995b). These phenotypic changes to morphology, physiology and behaviour could theoretically have both positive and negative effects on fitness. Compounding this is the current inability to reliably predict the variation in phenotype that will be produced by insertion of any single gene construct.

Based on the limited research that has been published to date, the Panel concludes that there is little, if any, empirical basis upon which one can reliably predict the outcome of interactions between wild and GM fish. On the one hand, the introduction of gene constructs can be associated with morphological and physiological changes to transgenic fish that may negatively affect the ability of transgenic fish to compete successfully with wild fish. For example, transgenic coho salmon appear to have reduced abilities to irrigate their gills (Devlin et al., 1995a), thus reducing their respiratory capabilities. They have also been reported to have reduced swimming

abilities (Farrell et al., 1997). By contrast, critical swimming speeds of growth hormone-enhanced Atlantic salmon appear not to differ from non-transgenic controls (Stevens et al., 1998), suggesting that transgenic Atlantic salmon would not be disadvantaged by reduced locomotory abilities. Increased ability by transgenic fish to compete for food (a positive effect on fitness), coupled with reduced vigilence to predators (a negative effect on fitness), has been suggested by two recent studies that have documented increased feeding rates by GM coho and Atlantic salmon in the presence and absence of non-transgenic conspecifics (Abrahams and Sutterlin, 1999; Devlin et al., 1999).

It is reasonable to predict that the threat to native populations posed by ecological interactions with either transgenic or non-transgenic fish will be greater for small populations than for large ones. In this respect, a small population may be numerically small, or it may be small relative to its historical abundance. Although it is the former characterization of small population size that is often of concern, the latter characterization may be of equal import.

Evaluating the Environmental Safety of Genetically Modified Fish

Experimental Facilities and Evaluation Protocol

Unlike many plants and terrestrial animals, it would be very difficult, if not unwise, to incorporate field trials in an evaluation process designed to assess the potential risks that genetically engineered fish might pose to native species. Once transgenic fish were placed into a natural ecosystem for a field trial (e.g. to compare growth rates and survival of juvenile transgenic and wild conspecifics), the probability of being able to then remove every transgenic fish from that lake or stream would be very low.

Nonetheless, under special circumstances, field trials of a sort could be undertaken in a facility, or even natural systems, devoted to such experimental study. One example of such an experimental facility would be a section of river or stream separated from the main stem of that river by barriers that would be impassable to fish and that would permit control of water flow through the experimental stream section (e.g. via stop-logs). However, while such a facility might allow one to evaluate potential risks to native riverine fish, it would be impractical to design a similar experimental facility in a lake, unless remote lake/river systems were designated as experimental systems solely for the study of the interactions between wild and transgenic fish, such as the Experimental Lakes Area established in Northwestern Ontario to study whole-ecosystem effects of pollutants and fishing (Schindler, 2001).

If one were to conduct field trials in such an experimental facility, they could comprise a suite of experiments conducted during the final stage of a tiered experimental protocol to evaluate the environmental safety of GM fish. During the first stage of such an approach, there are a number of experiments that could be conducted, each of which would be designed to evaluate the

probability that transgenic fish would negatively influence the population growth rate, and thus the persistence, of wild fish.

Following is a series of research questions that should be addressed when assessing the potential consequences of transgenic and non-transgenic cultured fish on the viability and persistence of wild fish. These can be grouped into four, non-mutually exclusive categories: genetic introgression, ecological interactions, fish health, and physical environmental health. These are broadly phrased questions that will apply to interactions between members of the same species as well as interactions between members of different species.

I. Genetic Introgression

1. What is the probability that cultured fish will reproduce with wild fish? Does this probability differ between sexes?
2. What is the probability that a transgenic fish will transmit its novel gene construct to offspring resulting from matings with other transgenic fish and with wild fish?
3. What is the range of pleiotropic effects on the phenotype that recombination of the novel gene construct might produce?
4. Is there a difference in the viability of offspring produced by crosses between cultured fish, pure wild crosses, and mixed crosses?

II. Ecological Interactions

In order of preference (i.e. increased similarity between experimental and natural conditions), experiments to address the following questions could be conducted in circular or longitudinal hatchery tanks, stream tanks or hatchery raceways, or experimental natural stream sections (as described above). Questions can be asked of offspring, notably during the juvenile stage, produced from matings (pure and hybrid) in the natural environment, and they can be asked of cultured fish that escape from aquaculture farms and enter the natural environment of wild fish.

1. Comparing cultured (pure and hybrid) and wild fish, are there significant differences in growth rate, survival, feeding rate, predator-avoidance behaviour, critical swimming speed, agonistic behaviour (e.g. aggression, territoriality), habitat selection, movement, migration, or dispersal?
2. Do escaped cultured fish compete with wild fish for food or space?
3. Do escaped cultured fish prey upon juvenile wild fish?
4. Do escaped cultured fish, sterile or not, negatively affect the reproductive success of wild fish (e.g. by nest superimposition, or by increased density on the spawning grounds)?

III. Fish Health

1. Is the disease and parasite profile of cultured fish likely to differ from that of wild fish?
2. What is the probability of disease/parasite transfer between cultured and wild fish?

IV. Changes to Environmental Health Effected by Aquaculture Farms

1. Do residues from factors such as antibiotics, high faeces concentration, vaccines and food accumulate near aquaculture sites and, if so, do they affect the microbial community in the bottom substrate?
2. Do aquaculture netpens serve as predator attractors, increasing predation risks to wild fish?
3. Do aquaculture farms influence the migratory behaviour of wild fish?
4. Do aquaculture farms allow for increased prevalence of diseases or parasites in cultured fish and, if so, does this increase the likelihood of their transmission to wild fish?

Density-dependent Effects and Population Viability

Critical to most of the questions posed above is the degree to which the consequences to wild population viability resulting from interactions between wild and cultured fish are likely to depend on density. Specifically, it is critical to note that the influence of cultured escapees on a wild population will depend on the number of farmed escapees, N_F, the size of the wild population of interest, N_W, and the size of the wild population relative to some conservation-based metric (i.e., $N_{(W|C)}$).

In a general sense, and in the absence of detailed experimental studies, the probability of negative consequences to the viability and persistence of a wild population effected by intrusions of escaped cultured fish can be assessed from the following table of population size inequalities.

Probability of Negative Consequences to Wild Population	Abundance of Cultured Population Relative to That of a Wild Population
Very High	$N_F > N_W < N_{(W\|C)}$
High	$N_F > N_W > N_{(W\|C)}$
Medium	$N_F < N_W < N_{(W\|C)}$
Low	$N_F < N_W > N_{(W\|C)}$

The table draws attention to the premise that cultured fish will be more likely to have a negative impact on wild fish when the number of escapees potentially interacting with wild fish exceeds the size of the wild fish population(s), particularly when the wild population(s) is itself small relative to some conservation-based metric of population size.

Sterility of Genetically Modified Fish

Induction of Triploidy

One widely discussed means of reducing potentially negative consequences of genetic interactions between wild and cultured fish is to render the latter sterile before they are placed in sea cages. If cultured fish can be made sterile, it would eliminate the potentially deleterious consequences of interbreeding between wild and cultured fish. (Based on data from five studies of transmission of growth-hormone gene constructs to F1 progeny in salmonids, consisting of 25 crosses from founder transgenic parents, Devlin (1997) estimated that the probability of transmission of novel genes from parents to offspring averages 15.6% $\pm$ 3.1%.)

The only effective method for mass producing sterility in most fish is to induce triploidy at the egg stage very early in development (Benfey, 1999). By exposing eggs to thermal or hydrostatic pressure shortly after fertilization, one can disrupt the normal movement of chromosomes during meiosis, essentially by making the eggs retain the second polar body (a package of maternal chromosomes which would normally leave the egg shortly after fertilization).

Triploid individuals, which possess three complete chromosome sets in their somatic cells, differ from conspecific diploids in three fundamental ways. Triploid fish are more heterozygous, they have larger although fewer cells in most tissues and organs, and their gonadal development is disrupted to some extent, depending on the sex (Benfey 1999). Females typically remain sexually immature, although Johnstone et al. (1991) reported a 0.1% rate of partial maturation in triploid Atlantic salmon. Triploid males, on the other hand, produce spermatozoa, exhibit normal spawning behaviour, and will mate with diploid females (Benfey, forthcoming). However, the development of offspring produced by matings between triploid males and diploid females is severely impaired, resulting in death during the embryonic and larval stages. Thus, all-female populations of triploids are better suited for aquaculture than mixed-sex, triploid populations.

Sterility as a Mitigative Tool to Minimize Potential Environmental Risks

In principle, triploidy would seem to be the ideal means of minimizing the potentially negative influences of interactions between cultured transgenic and non-transgenic fish and wild members of the same species. This is reflected by the DFO and International Council for the Exploration of the Sea (ICES 1995) endorsement of the recommendation that transgenic fish be permitted in aquatic netpens only if they are first rendered sterile.

However, there are three reasons why triploidy is unlikely to be an effective mitigative tool in the near future. These are based on the considerable uncertainty associated with the degree to which 100% sterility can be achieved in practice, the consequences of ecological interactions between triploid and wild fish, and the likelihood that the aquaculture industry would favour triploid fish over their diploid counterparts.

The effectiveness of the technology used to induce triploidy, and the incidence of sterility achieved, depends on a number of factors, perhaps most notably on the experience of the individual performing the technique (T.J. Benfey, Department of Biology, University of New Brunswick, pers. comm. 23 June 2000). Nonetheless, when undertaken properly, the technique can be quite effective. Benfey (unpublished data), for example, found that of 450 Atlantic salmon for which triploidy was induced by hydrostatic pressure, the ploidy of 17 (3.8%) individuals could not be ascertained and no individuals were confirmed as being diploid. Kapuscinski (2000) reports that triploidy can be successfully induced in more than 90% of offspring in large-scale production, noting however that this success rate will vary with fish strain, egg quality, the age of the breeding fish, and induction conditions. As a precautionary measure, even under ideal conditions, triploidy should always be verified (e.g. by flow-cytometric measurement of erythrocyte DNA content) among experimental fish before they are released into netpens (Benfey, 1999). Such individual screening is necessitated by the fact that variability in operator experience, induction conditions and biological factors, coupled with inevitable human error, will compromise the effectiveness of the sterility procedure.

However, the cost associated with confirming sterility in each fish before their transfer to netpens makes it unlikely that the aquaculture industry would find it economically worthwhile to rear triploid fish commercially as food. Additional disincentives to the industry include the greater mortality experienced by triploid fish and their higher incidence of morphological deformities, relative to diploid fish (O'Flynn et al., 1997; Benfey, forthcoming). These differences in mortality and morphology between diploid and triploid fish might be reduced if, and when, the optimal conditions for rearing the latter have been identified.

Notwithstanding the uncertainty associated with achieving 100% sterility and the present economic costs of rearing triploids, it is critical to determine the degree to which sterility would effectively mitigate potential negative consequences of interactions between wild and cultured fish. This is of concern from both a genetic and ecological perspective. Although sterile fish may not be able to transmit their genes, if their spawning behaviour is not severely impaired by triploidy, males in particular may be able to mate successfully, negatively influencing the fitness of affected wild individuals. From an ecological perspective, sterile fish require food and space. Given that triploid and diploid salmonids are behaviourally and morphologically similar in those aspects of non-reproductive behaviour that have received study (O'Flynn et al., 1997; O'Keefe and Benfey, 1997, 1999), it would seem reasonable to predict that escaped cultured fish will compete with wild fish for food and space, and that large cultured fish will prey upon smaller wild fish. Furthermore, there is no reason to believe that competition and predation would be restricted to members of the same species. The deleterious effects of exotic fish introductions on wild

populations throughout the world is ample demonstration that a fish need not reproduce with another to negatively affect the other's viability and persistence.

For some salmonids, it is possible that the reduced activity of reproductive hormones caused by sterility might suppress the migratory behaviour of affected individuals, reducing the probability that sterile individuals will enter rivers and interact with wild fish in those rivers. There is evidence, for example, that diploid coho salmon sterilized by androgen treatment have a very low probability of entering rivers from the ocean (e.g. Solar et al., 1986; Baker et al., 1989). Notwithstanding the need to verify such a hypothesis for triploid transgenic and non-transgenic individuals (Benfey, forthcoming), hormone-induced suppression of migratory behaviour may be of little consequence to salmonids that regularly enter rivers from the ocean in the absence of maturation (e.g. Arctic char, brook trout).

Nonetheless, if the incidence with which a wild population is exposed to escaped cultured fish is small, the number of escapees relative to the wild populations is low, and if the potentially affected wild populations are near their carrying capacities (or some other conservation-based metric of sustainability), then the influence of sterile cultured fish on wild fish populations is likely to be small.

Regulatory Implications

DFO National Code on Introductions and Transfers of Aquatic Organisms

The stated purpose of this proposed National Code (DFO, 2000b) is to establish scientific criteria for the intentional introduction and/or transfer of live aquatic organisms into Canada, between provinces and territories, and within provinces and territories. In Canada, the primary reasons for such introductions and transfers include the creation or maintenance of recreational angling fisheries and the rearing of fish for human food consumption. Predominant examples of the latter in Canada include the existing aquaculture netpen facilities for Atlantic salmon and rainbow trout on the east and west coasts. Because of rapidly developing research within the aquaculture industry and within academia, requests for fish transfers can be expected to increase as the industry expands its comparatively nascent efforts in the rearing of fish such as Arctic char, Atlantic cod (*Gadus morhua*), Atlantic halibut (*Hippoglossus hippoglossus*) and yellowtail flounder (*Limanda ferruginea*).

DFO Draft Policy on Research with, and Rearing of, Transgenic Aquatic Organisms

The motivation for this draft policy (DFO, 2000a) lies in the expectation that application for commercial production of a transgenic fish is imminent. Such an application was filed in the US in early 2000 for transgenic Atlantic salmon by a company (A/F Protein Canada) whose research laboratory is located in Prince Edward Island. In addition to fish, transgenic research has been

undertaken on marine invertebrates. Examples include the insertion of a growth hormone gene construct into abalone, a slow-growing mollusc, and the insertion of a marker gene into giant prawn (Canada, 1998), although there is no strong indication that transgenic marine invertebrates will be submitted for approval to CFIA within the decade. The draft policy is to be used on an interim basis, until specific Regulations are enacted under the Fisheries Act. Provinces may establish provisions and requirements additional to those set out in this national policy (DFO, 2000a).

Notably, the Draft Policy on transgenic fish makes the observation that, "because escape from commercial aquaculture cages and netpens has been significant, fish placed in them *must be treated the same as fish released into the natural ecosystem* (italics added)". Given the potentially negative consequences of introgression between transgenic and wild fish, the DFO has recommended that transgenic fish be sterilized before release into commercial aquatic rearing facilties. (A population is defined here as a group of potentially interbreeding individuals, found in a geographically limited area, that are members of the same species. Operationally, for fish, such a definition is often applied to conspecific individuals spawning in the same river or lake.)

Specifically, the DFO draft policy on transgenic aquatic organisms recommends that:

1. Initially, and until otherwise authorized, rearing of transgenic organisms outside a laboratory may be made only with functionally sterile organisms.
2. Requests to hold reproductively capable transgenic aquatic organisms in facilities such as dug-out, or by-pass natural or semi-natural ponds, netpens, etc., for broodstock development, or other purposes *may be considered in exceptional circumstances and will be subject to a public consultation* (italics added)".

The document does not explain why the sterility requirement should be applied only when transgenic organisms are *initially* introduced outside a laboratory, nor does it specify the "exceptional circumstances" under which releases of non-sterile transgenic fish would be considered.

Proposed Aquatic Organism Risk Analysis

The Implementation Guidelines of the DFO proposed National Code on Introductions and Transfers and of the Draft Policy on Transgenic Aquatic Organisms detail an Aquatic Organism Risk Analysis that must be completed for *most* new applications for introductions or transfers of fish. An organism may be deemed exempt from the Code, by the Minister, if the importation of that organism "presents minimal risk of negative impact on fisheries resources, habitat, or aquaculture". However, in the absence of a formal risk analysis, it is not clear how these exemptions would be justified scientifically.

Aquatic organism risk analyses are the responsibility of the DFO, unless the authorizing jurisdiction requires the risk analysis to be prepared by the proponent. The risk analyses for evaluating environmental consequences associated with the introduction of non-transgenic and transgenic aquatic organisms are identical, and consist of the following two parts, each of which comprises three steps:

Part I – [Transgenic] Aquatic Organism Ecological and Genetic Risk Assessment

Step 1: Determining the probability of establishment. The *Probability of Establishment* is subjectively assigned the category of High, Medium or Low. The *Level of Certainty* associated with this probability assessment is assigned one of the following categories: Very Certain (VC), Reasonably Certain (RC), Reasonably Uncertain (RU) or Very Uncertain (VU).

Step 2: Determining the consequence of establishment of an aquatic organism, with associated subjective estimates of *Consequences of Establishment* (High, Medium, Low) and *Level of Certainty* (categories are those given in Step 1).

Step 3: Estimating aquatic organism risk potential. The Final Risk Estimate is assigned a single probability rating (High, Medium, Low), with an associated level of certainty (VC to VU), based on the *Probability of Establishment* (Step 1) and the *Consequences of Establishment* (Step 2) assessments. The probability rating in assigning Final Risk is the higher of those delineated in steps 1 and 2; the level of certainty is that corresponding to the less certain of the two levels identified in steps 1 and 2.

Requirements for Approval: The requested Introduction or Transfer will be recommended for approval only if the overall estimated risk potential is Low and if the overall confidence level for which the overall risk was estimated is Very Certain or Reasonably Certain. However, the regional Introductions and Transfers Committees responsible for these evaluations can identify mitigative measures that would, in their opinion, reduce High and Medium risk potentials to a Low level. Possible mitigation measures identified by the Code include use of genetically similar stocks, sterilization, and use of containment facilities to prevent escapes.

Part 2 – Pathogen, Parasite or Fellow Traveller Risk Assessment Process

Step 1: Determining the probability of establishment, with associated subjective estimates of *Probability of Establishment* and *Level of Certainty* (see above).

Step 2: Determining the consequences of establishment of a pathogen, parasite or fellow traveller, with associated subjective estimates of *Consequences of Establishment* and *Level of Certainty*.

Step 3: Estimating pathogen, parasite or fellow traveller risk potential. The Final Risk Estimate is assigned a single value based on the *Probability of Establishment* (Step 1) and the *Consequences of Establishment* (Step 2), as described for the Ecological and Genetic Risk Assessment above.

Requirements for Approval: The requested Introduction or Transfer will be recommended for approval only if the overall estimated risk potential is Low and if the overall confidence level for which the overall risk was estimated is Very Certain or Reasonably Certain. However, the DFO's Introductions and Transfers Committees can identify mitigative measures that would, in their opinion, reduce the risk potential to a Low level. Possible mitigation measures include health inspection and certification, pre-treatment for pathogens, diseases and parasites, and vaccination, among others.

Critique of Current Regulatory Framework and Proposed Risk Aquatic Organism Analysis

CEPA (Canadian Environmental Protection Act)

Until the DFO establishes regulations pertaining to transgenic organisms in the Fisheries Act, the environmental consequences associated with the commercial rearing of transgenic aquatic organisms will be assessed under CEPA. According to CEPA Regulations, in order for regulators to assess the potential risks to the environment posed by transgenic animals, the proponent must provide information on "the potential of the organism to have adverse environmental impacts that could affect the conservation and sustainable use of biological diversity" (Regulation 5(c) of Sections 29.16 and 29.19, Schedule XIX).

Despite the apparent breadth of this requirement, based on the Guideline (4.3.5.3) accompanying this Regulation, and based on interviews with Environment Canada officials, the Panel concludes that CEPA Regulations have no explicit data requirements for information pertaining to the potential effects on conservation and biodiversity posed by GM animals. The Panel views this to be a significant weakness in the current legislation and concludes that the existing regulatory framework is ill-prepared, from an environmental safety perspective, for imminent applications for the approval of transgenic animals for commercial production.

Sterility of Transgenic Fish

Upon initial consideration, it would appear that DFO's position regarding sterility of transgenic fish is in accordance with that of ICES, whose *Code of Practice on the Introductions and Transfers of Marine Organisms* stipulates that GMOs be reproductively sterile prior to release (ICES, 1995). However, one should contrast this recommendation with that made by the Working Group of ICES that deals specifically with transgenic aquatic organisms. This Working Group, represented by scientists from Canada, the US and countries throughout northern Europe, recommended that:

> "Until there is a technique to produce 100% sterilization effectiveness, GMO[s] should not be held in or connection with open water systems" (ICES, 1997).

It is the opinion of this ICES Working Group that existing techniques for effecting sterility are not 100% effective (ICES 1997, 1998), an opinion with which the Expert Panel agrees. Given, then, that 100% sterility cannot be ensured, transgenic fish should not be placed in aquatic netpens.

DFO's Draft Policy recommendation that sterile GM fish be permitted in aquatic netpens does not appear to be shared by the North Atlantic Salmon Conservation Organization (NASCO), whose parties include Canada, Denmark (in respect of the Faroe Islands and Greenland), the European Union, Iceland, Norway, Russia and the US. (Subject to the approval of the Council, the Convention is open for accession by any State that exercises fisheries jurisdiction in the North Atlantic or is a State of Origin for salmon stocks subject to the Convention.)

The NASCO Guidelines on Transgenic Salmon (adopted by the Council) state that the Parties agree to "take all possible actions to ensure that the use of transgenic salmon, in any part of the NASCO Convention area, is confined to secure self-contained, land based facilities" (NASCO, 1997). This text is also reflected in the Revision to Protocols (paragraph 5.5 entitled Transgenic Salmon) of North American Commission document NAC(98)6, the Draft Discussion document for Revision to Protocols for the Introduction and Transfer of Salmonids (NASCO, 1998). It is worth noting, however, an internal inconsistency in the North American Commission document. In Section 2.2.1, the protocols state that "Transgenic salmonids may be used in marine or freshwater cages if they are reproductively sterile". It is somewhat strange that there should be a different approach between the Council's guidelines (agreed to by all Parties) and the North American Commission Protocols, although it should be borne in mind that the latter are still only in the form of a discussion document.

Aquatic Organism Risk Analysis

Despite its positive intent and potential breadth of information requirements, the Aquatic Organism Risk Analysis by which the DFO has proposed to evaluate the environmental safety of

transgenic aquatic organisms has one primary weakness: the probabilities and consequences of establishment by GM fish, and their associated certainty levels, are based on subjective evaluations supported by existing scientific literature and by the opinions of those who are members of DFO Introductions and Transfers Committees. There is no requirement for either the Proponent or DFO to undertake scientific analyses, or to collect experimental data, in support of the risk analysis process.

DFO (2000a, b) does note that:

> "The strength of the review process is not in the ratings but in the detailed biological and other relevant information statements that motivate them."

Herein lies a paradox. The Code states, in effect, that the strength of the review process is reflected by the biological data underlying the risk probability assessments. Logically, then, the *absence* of biological data pertaining to a specific introduction/transfer request must necessarily be associated with a *weak* review.

In addition, the proposed Aquatic Organism Risk Analysis should account for changes to risk associated with changes to the *population density* and *conservation status* of potentially affected organisms (see above). This "conditional" nature of potential consequences to wild population viability and persistence underscores the points that:

1. risks to environmental health must be assessed on a case-by-case and population-by-population basis,
2. that these risks should be reviewed regularly (e.g. every 5 years), and
3. that it would be inconsistent with the precautionary approach to assign general environmental risk probabilities that will be applicable to all environments in all parts of the country.

Given the paucity of scientific data and information pertaining to the environmental consequences of genetic and ecological interactions between cultured and wild fish, DFO's Aquatic Organism Risk Analysis, despite its laudable intentions, will be unable to provide strong, accurate, reliable assessments of potential risks to the environment posed by the introduction and transfer of GM fish. This dearth of comparative research, the difficulty (because of genotype by environment interactions) in being able to use laboratory research to predict environmental consequences reliably, and the unpredictable nature of complex pleiotropic phenotypic effects of gene insertions, lead the Expert Panel to conclude that it would be prudent and precautionary to impose a moratorium on the rearing of GM fish in aquatic facilities.

Future Research

Clearly, there is a need for research which addresses the consequences of interactions between wild and GM aquatic organisms. Examples of the questions that should be addressed by such research are identified earlier in this section. In this regard, the Canadian Biotechnology Strategy has recently provided support for research on transgenic Pacific salmon at DFO's West Vancouver Laboratory and on triploid salmonids by researchers at the University of New Brunswick. In addition, within the next 10 years, research on interactions between wild and cultured salmonids, funded by AquaNet, a National Centre of Excellence, will be undertaken.

These research initiatives will complement the comparative research that has been done to date on wild and GM fish, notably by Devlin and colleagues at DFO's West Vancouver Laboratory, but increasingly by academic scientists from various Canadian universities (e.g. Manitoba, Guelph, New Brunswick) in collaboration with industry. The greatest strength of this research lies in the scrutiny the work receives by the scientific community during the anonymous peer review of the manuscripts prior to publication and by continuous evaluation of the widely available published papers and associated data.

Public Perception of Environmental Risks Posed by Cultured Fish

One of the difficulties in assessing potential environmental risks posed by the introduction of cultured fish to natural ecosystems is the imprecise threshold of risk that different sectors of society are willing to accept. For some individuals, angling associations, aquaculture companies and government agencies, an absence of obviously negative consequences, even in the absence of relevant scientific studies to examine such consequences, appears to constitute evidence that cultured fish have negligible influences on wild populations. Indeed, depending on the individual or organization, the criterion for a negative influence effected by the presence of a cultured population probably ranges from any reduction in the abundance of a wild population to the commercial or biological extinction of a wild population.

The recreational fishing industry in Canada provides one example of the potential for extremely divergent perceptions of the potential environmental risks posed by cultured fish. Among the 92 fish species and 13 "forms" (subspecies, varieties, hybrids) identified by Crossman (1991) as having invaded Canadian freshwater lakes and rivers, 71 were authorized introductions (DFO, 2000b). Of the eight species of salmon and trout listed in the Government of Ontario's 2000 Recreational Fishing Regulations (Ontario, 2000), only three (two if one excludes the reintroduced Atlantic salmon) are native to Ontario and one (brown trout) is not native to Canada. In the late 1990s and in 2000, non-native fish, such as rainbow trout and brown trout, continue to be stocked into Ontario's lakes and rivers (OMNR, 2000). In fact, one of these intentionally

introduced non-native fish (splake) is actually an inter-specific hybrid produced by artificially breeding lake trout (*Salvelinus namaycush*) with brook trout.

Furthermore, the Government of Ontario, and presumably most angling associations, do not consider pink salmon, chinook salmon, coho salmon, rainbow trout, brown trout, or the inter-specific hybrid splake to be non-native species in Ontario — none of these three is identified as exotic species in the 2000 Ontario Fishing Regulations (Ontario, 2000). Oddly, of the 3 non-native fish that are mentioned, the rainbow smelt (*Osmerus mordax*) is actually native to eastern Ontario (Scott and Crossman, 1973).

Thus, to many sectors of society, cultured fish will be perceived to pose a threat to the environment only when they negatively influence the abundance of a commercially or recreationally exploited species.

RECOMMENDATIONS

The Panel concluded that there were significant scientific uncertainties associated both with the potential consequences of genetic and ecological interactions between transgenic and wild fish, and of the mitigative utility of rendering GM fish sterile in aquatic facilities. As a consequence, the Panel recommends that:

6.13. A moratorium be placed on the rearing of GM fish in aquatic netpens.

6.14 Approval for commercial production of transgenic fish be conditional on the rearing of fish in land-based facilities only.

6.15 Reliable assessment of the potential environmental risks posed by transgenic fish can only be addressed by comprehensive research programs devoted to the study of interactions between wild and cultured fish.

6.16 Potential risks to the environment posed by transgenic fish must be assessed not just case-by-case, but also on a population-by-population basis.

6.17 Identification of pleiotropic, or secondary, effects on the phenotype resulting from the insertion of single gene constructs be a research priority.

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7. SUBSTANTIAL EQUIVALENCE AS A REGULATORY CONCEPT

INTRODUCTION

One of the major challenges facing regulators of GM crop varieties world-wide has been deciding what comprises a meaningful difference between an existing crop variety and its GM derivatives. The genetic differences are apparently modest, and the GM derivatives retain most of the familiar characteristics of the parental variety, although the GM variety clearly possesses at least one additional novel (transgenic) trait. Regulators have generally taken the position that GM derivatives are so similar to the conventional varieties from which they have been derived that the two can be considered "substantially equivalent".

It is clear that GM varieties and conventional varieties are indeed very similar. However, application of this term to a new GM variety has become, within the present regulatory environment, effectively a declaration of safety. The validity of this use of "substantial equivalence" as a regulatory decision tool has become a hotly debated issue.

In this chapter, we explore the origins and applications of "substantial equivalence", and the basis of the debate. We also discuss what the Panel feels would be required to make this concept a valid metric for decisions regarding approval of new GM products.

THE ORIGINS OF "SUBSTANTIAL EQUIVALENCE"

The origins of "substantial equivalence" as an operational construct reside within the conventional breeding process. Plant breeders work primarily with highly refined breeding lines whose genetic heritage is known, and whose progeny have been evaluated from countless sexual recombination events. In effect, the existing gene pools are being shuffled into new combinations of alleles (the primary source of phenotypic variability) with additional variation often being created through incorporation of genetic material from distant relatives (wide crosses), and the ongoing appearance of spontaneous mutations in the genetic backgrounds in use. The expectation, borne out by years of successful crop variety development, is that "barley is barley is barley" (i.e. most, if not all, of the new gene combinations will produce a "barley" phenotype). Those that fail to meet that expectation are eliminated from the breeding program, and the most promising of the remaining lines are carried forward. The range of variability that appears in these progeny generations can be significant but, in general, such gene shuffling consistently recreates the same basic plant, and the expectation of "equivalence" has been fulfilled. The history of success in variety development through conventional breeding thus demonstrates that, despite occasional exceptions (Zitnak and Johnston, 1970; Hellenas et al., 1995), it is usually possible to recombine

genes within a species in many ways and create broadly similar, non-hazardous, phenotypic outcomes.

The caveat to this conclusion, however, relates to the relative genetic uniformity of the material used in most crop breeding programs. Selection over millennia for enhancement of desirable traits, and for absence of undesirable properties, has converted most of our major crops into genetically homogeneous forms which have lost most, if not all, of their capacity to either harm consuming organisms or compete successfully outside of a managed agro-ecosystem. Given this general "disarming" of the original species, it is perhaps not surprising that shuffling of the remaining functional genes within contemporary breeding programs can be routinely undertaken without creation of harmful progeny.

HOW HAVE NEW CROP VARIETIES NORMALLY BEEN APPROVED?

For crop varieties developed through conventional breeding, the testing required for new genotypes being considered for commercial release follows a long-established model. The conventional crossing and selection process through which new varieties are produced will, by design, have created new gene combinations. At the level of the genome, such new combinations may involve only modest local differences in DNA sequence in comparison with existing varieties, but these small differences are likely to be numerous, and distributed non-uniformly across the genome. Their collective effects will be responsible for generation of the new phenotype, conditioned to some extent by interactions with the environment.

However, no straightforward method has been available for assessing, *a priori*, the specific contribution of each genetic difference to the new phenotype. It is therefore accepted practice to compare directly any new genotypes with existing varieties (referred to as "test" or "check" varieties) and to establish that the new candidate meets or exceeds specific standards for quality and performance. Such testing typically includes laboratory evaluation (e.g. chemical analysis) of the harvested plant parts, as well as comparative field performance data from test plots grown at multiple sites over a number of years.

Traditional breeding has frequently produced new crop varieties distinguished by possession of "novel traits", including greater herbicide tolerance, increased disease resistance, different seed colour, altered oil profile, etc. Where a novel trait accompanies the new genotype, the validity and stability of this specific trait will also be monitored under field conditions. However, interactions of such a breeding-derived trait with other parts of the genome are assumed either to be of no functional significance, or, should a negative impact perchance be created, to be readily detected during the usual field testing.

It should be noted that tests for direct human impacts such as toxicity or allergenicity would not normally be included in such routine variety evaluation, unless there were a prior history of problems of this nature associated with the species in question (e.g. glucosinolates in canola, glycoalkaloid accumulation in potatoes). Otherwise, the implicit assumption behind this methodology is that, even where a breeding-derived novel trait is involved, *new combinations of existing genes operating within highly selected germplasm are not expected to generate harmful outcomes*. In other words, while the new variety will not be identical to existing germplasm (otherwise no improvement would have occurred), it does meet the expectations for the crop in question, and offers some enhancement of one or more traits.

How Have Transgenic Crops Been Treated in This Context?

When faced with the question of what testing should be required for new genotypes that result from genetic engineering of existing crop varieties, regulatory agencies in Canada and elsewhere have invoked a line of reasoning that tries to mirror the historical practice in conventional breeding. In the case of transgenic material, the assumptions implicit in the conventional breeding methodology have been made explicit by rolling them up in the term "substantial equivalence". This concept was first described in a 1993 report from the Organisation for Economic Co-operation and Development (OECD), in which "substantial equivalence" was suggested as an operational mechanism to indicate that a GM organism was essentially similar to its traditional counterpart. The major conclusion of the OECD report was: "If a new food or food component is found to be substantially equivalent to an existing food or food component, it can be treated in the same manner with respect to safety". Subsequently, the World Health Organization (1995) published a report in which the concept of "substantial equivalence" as a decision threshold was promoted as the basis for safety assessment decisions concerning GMOs.

How Well Has "Substantial Equivalence" Been Accepted?

The adoption of "substantial equivalence" as a decision threshold has been criticized because of the ambiguity and lack of specificity of the term. The failure to define "substantial equivalence" clearly was emphasized by Millstone et al. (1999), who also stated that the "biotechnology companies wanted government regulators to help persuade consumers that their products were safe, yet they also wanted the regulatory hurdles to be set as low as possible". Those using the concept as a screening tool immediately defended "substantial equivalence", as shown by the subsequent correspondence to the journal *Nature Biotechnology*. For instance, Miller (1999) wrote that: "Substantial equivalence is not intended to be a scientific formulation; it is a conceptual tool for food producers and government regulators, and it neither specifies nor limits the kind or amount of testing needed for new foods".

Reflecting this ongoing uncertainty, the Committee on Food Labelling (February 2000) of the Codex Alimentarius, created by the Food and Agriculture Organization and the World Health Organization, decided to remove the term "substantial equivalence" from its draft recommendations for food and food ingredients obtained through modern biotechnology. This commission had already made the decision to delete the word "substantial" in 1999, and in 2000, proposed to use such phrases as "no longer equivalent" or "differs significantly" in the text of its recommendations. It was suggested that "if the nutritional value of a food or food ingredient is no longer equivalent to the corresponding food or food ingredient", certain conditions would apply, such as informing the consumer of a changed nutrient content. However, this negative approach to "equivalence" appears to constitute a rejection of the concept of "substantial equivalence" altogether, rather than a redefinition of it. The Codex ad hoc task force on Foods Derived from Biotechnology acknowledged this in its report of March 2000: "While recognizing that the concept of substantial equivalence was being used in safety assessment, several delegations and observer organizations stressed the need for further review of the concept and its applicability to safety assessment".

The Role of the "Substantial Equivalence" Concept in the Canadian Regulatory Process

In practice, the designation of a candidate GM crop variety as "substantially equivalent" to other, non-GM, varieties essentially pre-empts any requirement in Canada to assess further the new variety for unanticipated characteristics. Thus, the Decision Documents issued by CFIA in approving new GM canola crops for commercial release state: "Unconfined release into the environment, including feed use... but without the introduction of any other novel trait, is ... considered safe". Both in Canada and elsewhere, therefore, "substantial equivalence" is currently employed as an explicit rule stating the conditions under which it can be assumed that a new crop poses no more risks than a counterpart that is already considered safe. It represents one of the early criteria to be met in the regulatory decision trees (see Chapter 3). If a plant or food is judged to be substantially equivalent to one present in the Canadian diet, passage of this step in the decision tree spells success for its approval. Conceptual and practical implementation of "substantial equivalence" is thus the most critical element in the current approval process.

"Novelty" Versus "Equivalence"

The "substantial equivalence" concept is clearly rooted in the existing paradigm for new crop development through traditional methodologies. A breeder who has genetically manipulated a crop through crossing/selection takes it as a given that, despite the numerous small changes introduced into the genome of the new genotype, the species as an entity remains largely

unmodified. The new variety is thus assumed to be "substantially equivalent" to other varieties of the same crop. It is worth emphasizing that this assumption applies even if "novel trait" genes have been introduced into the breeding lines at some point, through use of wide crosses or mutation.

On the face of it, however, there would appear to be an intrinsic contradiction between the presence of "novelty" in a new plant genotype and a designation of "equivalence". This tension is reflected in the Seeds Regulations (under the administration of CFIA) which state that a "novel trait" introduced into cultivated seed "….[is one that] is not substantially equivalent, in terms of its specific use and safety for both the environment and for human health, to any characteristic of a distinct, stable population of cultivated seed of the same species in Canada, having regard to weediness potential, gene flow, plant pest potential, impact on non-target organisms and impact on biodiversity." Also, in the Feeds Regulations, a novel trait introduced into an animal feed is similarly described as making the feed no longer substantially equivalent to similar feed without that trait. It is clear that this treatment of novel traits recognizes their potential to create a human or environmental health hazard, and that a designation of "substantial equivalence" would only be justified if and when a novel trait can be demonstrated to have no safety implications "…for both the environment and human health…".

This framing of "substantial equivalence" links it intimately with the definition of "novel trait" in a way that leads to a logical impasse. If a "novel trait" can be demonstrated to have no safety implications "…for both the environment and human health…", the above description implies that the genotypes being compared must be "substantially equivalent" and that there is, in fact, no "novel trait" at issue. Conversely, if two genotypes are deemed to be "substantially equivalent" then no "novel trait", as defined above, can be present. The current language is thus unhelpful when it comes to describing the outcomes of transgenic variety evaluation.

This logical confusion is part of a larger ambiguity in the use of "substantial equivalence" in the regulatory world. The ambiguity can be seen in the original OECD formulation of the concept: "If a new food or food component is found to be substantially equivalent to an existing food or food component, it can be treated in the same manner with respect to safety". This can be interpreted in very different ways.

In one interpretation, to say that the new food is "substantially equivalent" is to say that "on its face" it is equivalent (i.e. it looks like a duck and it quacks like a duck, therefore we assume that it must be a duck — or at least we will treat it as a duck). Because "on its face" the new food appears equivalent, there is no need to subject it to a full risk assessment to confirm our assumption. This interpretation of "substantial equivalence" is directly analogous to the reasoning used in approval of varieties derived through conventional breeding. In both cases, "substantial equivalence" does not function as a scientific basis for the application of a safety standard, but

rather as a decision procedure for facilitating the passage of new products, GM and non-GM, through the regulatory process.

However, the OECD maxim cited above can be interpreted in quite a different manner, with the consequence that the need to establish scientifically that the new food is identical in its health and environmental impacts to its conventional counterpart is not so readily circumvented. This interpretation requires a scientific finding that the new food does not differ from its existing counterpart in any way other than the presence of the single new gene and its predicted phenotypic change. In every other way, phenotypically and in terms of its impacts on health and the environment, it will have been demonstrated to be identical to the existing food. Once this finding is made, the food can then be considered (i.e. "treated as") safe, in as much as the existing food is already considered safe, with the caveat that the phenotypic expression of the added novel gene(s) must also be demonstrated to have no negative health or safety impacts. In this interpretation, the concept of "substantial equivalence" functions as a scientific finding or conclusion that in turn becomes the justification for an assumption of safety. In effect, "substantial equivalence" is invoked as a standard of safety.

"Substantial equivalence" is commonly used by government regulatory agencies under the first interpretation, although public statements defending the use of the concept often play upon its inherent ambiguity by suggesting the second interpretation. The CFIA Schematic Representation of the Safety Based Model for Regulation of Plants (Chapter 3) demonstrates how initial findings of "familiarity" and "substantial equivalence" are used to exempt new plants from the third step, which is the full environmental safety assessment. Step 2.1 in the schematic requires that scientific data and rationale support any conclusions that the new plant "will not result in altered environmental interaction compared to its counterpart(s)". The question that concerns the Expert Panel is whether in actual practice these conclusions are based upon a full analysis of the new organism in question, or whether they are based upon unsubstantiated *assumptions* about the equivalence of the organisms, by analogy with conventional breeding. We have concluded that the latter is a consistent reading of the schematic, and is what often occurs in practice.

In summary, the Panel has identified two different uses of the concept of "substantial equivalence":

1. A GM organism is "substantially equivalent" if, on the basis of reasoning analogous to that used in the assessment of varieties derived through conventional breeding, it is assumed that no changes have been introduced into the organism other than those directly attributable to the novel gene. If the latter are demonstrated to be harmless, the GM organism is predicted to have no greater adverse impacts upon health or environment than its traditional counterpart. We refer to this interpretation as the *decision threshold* interpretation.

2. A GM organism is "substantially equivalent" if rigorous scientific analysis establishes that, despite all changes introduced into the organism as a result of the introduction of novel genes, the organism poses no more risk to health or to the environment than does its conventional counterpart. We refer to this interpretation as the *safety standard* interpretation.

The Expert Panel accepts the validity of the concept when used in the "safety standard" interpretation. We have grave reservations about its validity when employed in the "decision threshold" interpretation.

In the Panel's view, the use of "substantial equivalence" as a decision tool within the regulatory process would appear to demand a careful assessment of safety impacts associated with any "novel trait" being considered for deployment in a new transgenic variety. If the presence of the novel trait can be rigorously demonstrated to be harmless (or the harm does not surpass a certain agreed-upon threshold) in the tested genetic/environmental context, the new genotype can be considered to be as safe as the original variety from which it was derived during the genetic engineering process. The question then becomes one of defining "rigorous demonstration" and its implementation.

HOW DO THE PRODUCTS OF GENETIC ENGINEERING DIFFER FROM THE CONVENTIONALLY DERIVED PRODUCTS?

The current generation of GM crops differs in its genetic origins from crop varieties created through conventional breeding. Unlike the mixture of parental genes represented in a conventionally derived variety, a first-generation GM crop is distinguished from its parental variety by the incorporation into that original parental genome of a novel single gene trait. In the GM crops presently in production, these traits are controlled by gene sequences derived almost exclusively from non-plant sources (i.e. bacterial, viral or insect DNA). It has been pointed out that the resulting phenotypes may be functionally similar to naturally occurring examples of analogous genetic traits, such as herbicide, insect or virus disease tolerance. Nevertheless, there is little serious debate about the fact that the presence of any of these transgene DNA sequences in a GM crop variety represents an example of incorporation of a "novel trait".

The fact that the "novel trait" is being controlled by a tract of DNA that makes up only an extremely small part of the plant genome, and that its introduction into the plant genome was not accompanied by transfer of large numbers of other genes (or, more accurately, other alleles) physically associated on the same chromosome, as would happen in conventional breeding, has led to genetic engineering for novel traits being characterized as "more precise".

WHAT ARE THE ANTICIPATED CONSEQUENCES OF "PRECISE" SINGLE GENE MODIFICATIONS?

In the very simplest model, the term "precise" implies that the only changes resulting from such a genetic modification should be:

- presence at a defined site within the genome of one small novel stretch of DNA;
- expression of one new mRNA encoded by the inserted gene;
- expression of a new protein translated from the new mRNA, when the transgene encodes a protein;
- appearance of a new catalytic activity displayed by that protein (if the protein is an enzyme); and
- changes in the pools of relevant metabolic substrates/products affected by that catalysis in the transgenic tissues.

In other words, this linear sequence of outcomes would be predicted to occur without significantly perturbing the remaining transcriptional, translational and metabolic activities in the plant. The genotype and phenotype of the genetically engineered variety will thus differ from that of the original variety from which it was derived *solely* in terms of the "novel trait" represented by the transgene and its products. In all other respects, the transgenic variety will be identical to that parental variety. If this simple linear model is valid, the evaluation of the transgenic variety need only focus upon the *predicted* phenotypic characteristics conferred by the transgene, and their potential to cause harm. If that narrowly focused evaluation finds no grounds for concern, the transgenic variety can be considered "equivalent" to existing varieties because the genetic background within which the transgene is operating is identical to that of one of those existing varieties.

IS THIS SIMPLE LINEAR MODEL VALID?

As outlined above, the primary assumption operating within this simple linear model is that the action of one gene and its products will have no significant effects on other genes, gene products or metabolic functions in the tissues within which it is expressed. However, empirical evidence suggests that linear models are not good predictors of complex biological systems, which involve extensive interactions between cellular components at all levels. While our understanding of the intricacies of genetic interaction networks is still only poorly developed, it is clear that living cells are exquisitely tuned to both their internal and external environments. Perturbations in either will typically induce a spectrum of changes in gene expression, protein synthesis and metabolic patterns, all designed to enhance the organism's ability to survive and thrive. Mutations in single genes have long been known usually to produce multiple effects (pleiotropic effects) within the mutated organism. Even when visual assessment detects no differences between mutant

and wild-type forms, more detailed chemical analysis may reveal marked alterations in metabolism (Flehn et al., 2000).

The default prediction for the impacts of expression of a new gene (and its products) within a transgenic organism would therefore more logically be that this expression will be accompanied by a range of collateral changes in expression of other genes, changes in the pattern of proteins produced and/or changes in metabolic activities (Chavadev et al., 1994; Fischer et al., 1997; Burton et al., 2000; Eriksson et al., 2000; Flehn et al., 2000; Roessner et al., 2000). This is graphically demonstrated in the range of phenotypes displayed by transgenic salmon carrying a transgene encoding human growth hormone (see Chapters 5 and 6) or aspen trees expressing a transgene encoding a plant hormone modifier (Eriksson et al., 2000). It is, in fact, an accepted part of the process of genetic engineering of plants to screen for unusual phenotypes within the primary populations of transgenic crops generated in the laboratory (Matzke and Matzke, 2000). These will usually be discarded and only those lines displaying apparently normal phenotypes will be carried through for further analysis and/or breeding.

A related prediction, based on our appreciation of the complexity of biological systems, is that the nature of any such transgene-related changes is likely to be conditioned by:

- the genetic background within which the new gene is being expressed;
- the developmental and physiological status of the transgenic organism; and
- the environmental pressures impinging upon it.

In other words, an altered phenotype may only appear at a particular growth stage, or in response to specific environmental conditions.

It is important to recognise, however, that most, if not all, of these induced changes may be quite minor. In addition, biological systems are remarkably robust and flexible. The induced changes may therefore be readily accommodated within the normal dynamic range of cellular activities without apparently affecting the phenotype (Flehn et al., 2000). Nevertheless, the conclusions relevant to this discussion are that:

- unanticipated changes can be induced by expression of a novel gene; and
- their phenotypic consequences need to be assessed empirically across time and environments.

If unanticipated changes are likely to have been induced by transgene insertion, how might these be tracked, and how could their significance be assessed, in the context of a regulatory approval process?

ASSESSING THE SIGNIFICANCE OF DIFFERENCES

The obvious approach to analysis of the consequences of the presence of the transgene is to employ direct testing for harmful outcomes. In the case of food or feed products, this would mean testing for short-term and long-term human toxicity, allergenicity or other health effects (see Chapter 4). The environmental impacts of both local and landscape-scale deployment of the transgenic organism would also be assessed, over time and across relevant sites (see Chapter 6). At the end of this comparative analysis, an assessment must be made of the extent to which the transgenic variety deviates from the parental genotype, and whether any observed deviations are biologically significant. The absence of significant deviations would remove any regulatory barrier to variety approval (i.e. the transgenic variety would qualify as "substantially equivalent").

This approach has the obvious merit of directly addressing the potential for harm, which is the primary motivation for the regulatory process, and from that perspective it must remain the cornerstone of the approval process. To some extent, it represents the model followed within the current Canadian regulatory system. However, this empirical approach presents some serious challenges.

First, the integrity of the final assessment is obviously dependent on the depth and rigour of the testing regimes implemented. Inadequate, inappropriate or improperly conducted tests inevitably compromise the validity of the conclusions, while the determination of any "significant deviation" needs to be based on sound science, appropriate statistical analysis, and reliable baseline data, a resource which is not always available for a given trait, species and/or ecosystem. Concerns of this nature have been voiced about the current Canadian regulatory process (Barrett, 1999), and the lack of transparency in that process makes it difficult to establish how valid such concerns might be. Recommendations for the design and execution of suitable testing regimes, and the need for appropriately focused research programs, have been presented in other chapters of this Report, while the necessity for greater transparency has been discussed in Chapter 9.

BUILDING BETTER EVALUATION CAPACITY

While empirical screening directly addresses the immediate needs of the regulatory system, by itself it creates little opportunity for improving our understanding of the ways in which transgenes affect phenotype. Unless we learn more about the ways in which a transgene has modified the inner workings of the transgenic organism, it is difficult to develop any predictive capacity that would allow informed judgments about the likely performance of similar transgenes in other genetic or environmental contexts. In the absence of improved knowledge, testing of future novel genotypes must inevitably remain a largely empirical process, with all the associated complexity and costs.

It would therefore be highly desirable to integrate empirical screening with detailed analysis of the molecular and cellular status of the transgenic organism. These analyses should draw upon the "full system" molecular tools now being developed for our major crop species. The first complete plant genome DNA sequence (Arabidopsis) is now available, and the first cereal genome sequencing project (rice) is also in its final stages. These resources are largely the result of major international public research efforts whose output is freely available. However, agbiotech companies have also invested heavily in this area, creating large proprietary genomic databases for crops that are of specific interest to them (e.g. maize, potato, wheat).

While these public and commercial resources are still incomplete, they point to a not-too-distant future when a detailed knowledge of the genome and proteome of each of our major food crops will be available as a routine research tool. With these tools in hand, it should be possible to define accurately the structural and functional differences between any two genotypes within a crop species at four levels.

Level One - DNA Structure

In the case of a transgenic versus non-transgenic comparison, it should be feasible to establish unequivocally the location, size and nature of any insertion of a novel gene and to verify whether any additional changes (e.g. somaclonal variation) have been induced at the DNA level during the process of developing the transgenic genotype.

Of particular interest in this regard would be evidence that the transgene insertion has disrupted either a gene coding region, or associated regulatory regions. Examination of the phenotypic consequences of such insertion events would be part of the overall assessment of the transgenic genotype, and these data would also provide useful insights into biological function of discrete regions of the crop genome. It is noteworthy that a more detailed examination of the DNA sequence structure in *Roundup Ready* soybean varieties that had been developed by Monsanto almost a decade ago recently revealed the presence of short, extra stretches of transgenic DNA. These unanticipated insertions had not been detected in the original evaluation and approval process, and their impact, if any, on the transgenic phenotype is uncertain (Palewitz, 2000).

Level Two - Gene Expression

Knowledge of the exact structure of the transgenic organism's genome provides a concrete measure of the difference between the transgenic genotype and the parental variety from which it was derived. However, this knowledge does not, in itself, enable a prediction of phenotypic differences. Those differences will become manifest at the "downstream" levels of gene function, beginning with the expression of transcripts.

Thousands of genes are being expressed in a plant in an orchestrated manner at any given time. The rate and timing of transcript expression from any given gene in any particular cell represents an integrated response to many factors, internal and external, that impinge on that cell. The pattern of expression of all transcripts is thus an exquisitely sensitive monitor of cell and tissue status. In species where the effort has been made to assemble a complete set of the potential transcripts from the genome, it is possible to physically array DNA derivatives of these transcripts on high-density microarrays. The arrays can then be interrogated by hybridization with mRNA preparations derived from the plant tissues to be compared, and the identity and relative abundance of each transcript in each preparation assessed (Schenk et al., 2000; Wang et al., 2000). Carefully controlled DNA microarray analysis has the potential to reveal significant shifts in the overall pattern of gene expression associated with transgene insertion. For genomes that have been fully sequenced, other technologies can also provide a quantitative read-out of gene expression patterns (Velculescu et al., 1995).

The simple linear model discussed earlier predicts that only one new transcript will be detected in the transgenic line. However, should more extensive changes in transcript profiles be detected, microarray analysis immediately provides crucial information on the identity of the specific genes whose output is being affected. Knowledge of the biological role of those genes will allow a first estimate of the area(s) of metabolism or development in which a phenotypic change might be anticipated, and would thus help to focus the evaluation of the transgenic material on the most relevant issues. More sophisticated transcript profiling might explore differences on a tissue or organ basis, make comparative measurements over developmental time, and examine the interaction with different environments, all of which would improve the resolution and value of the resulting information (Aharoni et al., 2000).

Level Three - Protein Profiling

While the usual processing of genetic information predicts that a functional transcript will be translated to yield the corresponding protein, this correlation is neither perfect nor quantitative. Therefore, not all changes in gene transcript level in a particular cell will necessarily be reflected in predictable changes in the constellation of proteins synthesized and accumulated in that cell. Given that uncertainty, it would be desirable to determine whether transgene insertion has created any significant changes in the protein complement of the transgenic line, particularly since the great majority of food allergens are protein-based.

A comparative "proteomic" analysis of different plant tissues is a technically far more challenging exercise than transcript profiling. The methodologies available until recently have been limited in their throughput, reliability and sensitivity. However, new mass spectrometry-based

techniques show promise of being able to distinguish differences between very small samples of complex protein mixtures (Oda et al., 1999; Gygi et al., 1999). As these techniques are further refined, we can anticipate being able to create detailed and quantitative protein "fingerprints" for the same range of tissue samples that would be examined for transcript differences (Natera et al., 2000). Any novel proteins can be identified by mass spectrometry sequencing combined with database searches. Most importantly, perhaps, recombinant versions of such proteins can then be produced in substantial quantities as pure proteins, which would allow thorough testing for their potential allergenicity or anti-nutritive activity in humans and animals.

Level Four - Metabolic Profiling

Changes in transcript profiles and protein accumulation in a tissue will often be reflected in altered metabolite profiles. Of particular concern in plants is the potential for induced alterations in their secondary metabolite patterns. Most plant-derived toxicity problems are associated with accumulation in the plant tissues of unusual species-specific metabolites. These "secondary" metabolites represent an extraordinarily rich chemical arsenal that enables plants to survive as immobile organisms in a challenging environment. Since many of these chemicals render plants unpalatable or even toxic, it is not surprising that one of the outcomes of crop breeding over the centuries has been to suppress much of the original secondary metabolic output. However, secondary metabolism is highly plastic, and changes in enzyme levels and/or input metabolite availability can have a marked effect on the final metabolite profile (Bate et al., 1994). It would therefore be important to establish that transgene insertion has not significantly altered the secondary metabolite profile of the food tissues, or that, if such changes have occurred, these are not associated with increased risks to human, animal or environmental health (Firn and Jones, 1999). The basic technology for such an analysis is already available in the form of various chromatographic methodologies (HPLC and GC operating with a range of detector modes) (Roessner et al., 2000; Flehn et al., 2000). This would complement the standard "proximate analysis" which is used to assess the content of major nutrients in new foodstuffs.

CAN "SUBSTANTIAL EQUIVALENCE" BECOME SCIENTIFICALLY RIGOROUS?

The integrated approach suggested above would see newly developed transgenic genotypes subjected to intense scrutiny at six relevant levels (genome, transcript, protein, metabolite, health impacts, environmental impacts) before they were approved for commercial production. The answers obtained from the molecular analyses, in particular (Levels 1 to 4 above), would speak directly to the validity of the simple linear model of "precise" genetic engineering. If these analyses are conducted on a range of existing transgenic varieties and the predictions of the simple linear model prove to be valid, that outcome would provide essential

scientific support for the current regulatory view that the insertion of the transgene(s) has created no significant changes in the original variety, other than those predicted and desired. If, on the other hand, the molecular analyses demonstrate that the simple model is not valid, the data would provide immediate entry points for studying the impacts of the detected changes on human health and the environment. The outcome of those follow-up studies will then help determine whether the impacts create a significant risk.

The integrated approach would also enable the development of a better understanding of how genomes and their variants control phenotypes at many different levels. By carefully examining the environmental performance of transgenic organisms and correlating this with the activity and responses of the modified genome, a much more sophisticated understanding of the genotype/phenotype/safety linkage will be developed for each of our major food crop species. This cumulative experience will eventually allow more accurate predictions of trait expression, ecological fitness and potential risk, and thereby support reliable, *a priori* assessments of "substantial equivalence" with reduced levels of empirical testing. In the Panel's view, the goal should be to move away from an assumption of "precise" genetic engineering to a knowledge-based precise analysis of the resulting transgenic organisms.

Implementation of such an integrated evaluation process would initially increase the cost of GM variety approvals. The new "full systems" technologies are expensive (although these costs are expected to decline as capacity increases), and appropriate tools and robust protocols need to be developed, refined and implemented for each major Canadian crop. Baseline ecological studies across our major crop production areas and adjoining unmanaged ecosystems also need to be undertaken. However, these development costs should be regarded as a necessary long-term investment, both in the future of the major Canadian crop systems and in the genetic technologies capable of adding value to them. The Panel notes that the recent federal funding provided to create a national genomics initiative in Canada (Genome Canada) is a positive step in this direction.

RECOMMENDATIONS

7.1 Approval of new transgenic organisms for environmental release, and for use as food or feed, should be based on rigorous scientific assessment of their potential for causing harm to the environment or to human health. Such testing should replace the current regulatory reliance on "substantial equivalence" as a decision threshold.

7.2 Design and execution of the testing regimes should be conducted in open consultation with the expert scientific community.

7.3 Analysis of the outcomes of these tests should be monitored by an appropriately configured panel of "arms-length" experts from all sectors, who report their decisions and rationale in a public forum.

7.4 Canada should develop and maintain comprehensive public baseline data resources that address the biology of both its major agroecosystems and adjacent biosystems.

7.5 Canada should develop state-of-the-art genomics resources for each of its major crops, farm animals and aquacultured fish, and use these to implement effective methodologies for supporting regulatory decision making.

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8. THE PRECAUTIONARY PRINCIPLE AND THE REGULATION OF FOOD BIOTECHNOLOGY

INTRODUCTION

The Precautionary Principle has become a widely invoked doctrine within the field of risk regulation around the world. Though widely invoked, it is equally widely disputed and interpreted (Anon, 2000). Its roots are in the environmental movements of the 1970s, where it arose as part of a growing scepticism about the ability of scientific risk assessment and management models to predict accurately the adverse consequences of complex technologies (McIntyre and Mosedale, 1997). In essence, the principle advises that, in the face of scientific uncertainty or lack of knowledge, it is better to err on the side of protecting human and environmental safety than to err on the side of the risks (i.e. "Better safe than sorry.") (Barrett, 1999).

The Precautionary Principle has been the focus of much of the debate associated with biotechnology, as with other technological developments. Its proponents view it as a proactive and anticipatory approach to technology development essential to protecting human, animal and environmental health from potentially catastrophic harms that even the best science cannot always foresee (Gullett, 1997; Barrett, 1999). Its opponents view it as an unscientific attitude that seriously inhibits economic and technological development on the basis of unfounded fears (Miller and Conko, 2000). For example, it has been suggested that the recent adoption of the principle in the Cartagena Protocol on Biosafety (see below) has the potential to "lead to arbitrary unscientific rejection of some products" (Mahoney, 2000).

CURRENT STATUS

Since its introduction in European environmental policies in the late 1970s, the Precautionary Principle has emerged as one of the principal tenets of international environmental law (Shipworth and Kenley, 1999). Today, the principle is contained in over 20 international laws, treaties, protocols and declarations (Barrett, 1999), including the Protection of the North Sea (1984), the Montreal Protocol (1997), The Bangkok Declaration on Environmentally Sound and Sustainable Development in Asia (1990), The Climate Change Convention (1992), the Rio Declaration (1992), The European Union's Maastricht Treaty (1994), and The Fish Stocks Agreement (1995, signed by over 100 countries) (McIntyre and Mosedale, 1997; Barrett, 1999). It has also been considered by the International Court of Justice (e.g. the case of New Zealand challenging France on nuclear tests, Hungary's challenge to the Czech Republic regarding the Danube Dams Project, and in Ireland's case against the UK regarding the risk of radioactive material entering the marine environment [the "NIREX" case]) (McIntyre and Mosedale, 1997).

While the principle is not widely accepted in US law, American courts have upheld government regulatory decisions which are “precautionary like” (*Cellular Telephone Co.* v. *Town of Oyster Bay*, 166 F.3d 490, 494 (2d Cir. 1999) (Foster et al., 2000).

The Precautionary Principle has also been enshrined in international agreements affecting the regulation of plant and animal biotechnology in trade. For example, the principle is included in the Cartagena Protocol on Biosafety (agreed to in Montreal, January 2000). The treaty allows countries to use the Precautionary Principle to refuse import of GE food products. Article 11.8 states:

> “Lack of scientific certainty due to insufficient relevant scientific information and knowledge regarding the extent of the potential adverse effects of a living modified organism on the conservation and sustainable use of biological diversity in the Party of import, taking also into account risks to human health, shall not prevent that Party from taking a decision, as appropriate, with regard to the import of that living modified organism intended for direct use as food or feed, or for processing, in order to avoid or minimize such potential adverse effects.”

However, because the treaty later states that a rejection must be based on “credible scientific evidence,” the exact impact of the treaty remains unclear (Helmuth, 2000). This proviso reflects a central unresolved issue in national and international invocations of the principle — namely, the issue of what level of scientific evidence of potential harm is required to trigger the application of precaution.

The 1992 United Nations Conference on Environment and Development (The Rio Declaration) adopted language similar to the Cartagena Protocol. Principle 15 states that “Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation.” The Rio and Cartagena formulations are widely cited as definitive statements of the Precautionary Principle by both supporters and critics.

CONTROVERSIES SURROUNDING THE PRECAUTIONARY PRINCIPLE

As noted above, the Precautionary Principle has been the subject of much debate. Despite a substantial amount of political support throughout the world, the principle has attracted much criticism. Some of the more commonly heard criticisms of the principle include the following:

1. The Precautionary Principle lacks a uniform interpretation (Barrett, 1999). One study found 14 different interpretations of the principle (Foster et al., 2000). Some treaties, such as that of the European Union, refer to the Principle but do not actually define it. Other international instruments, such as the Cartagena Protocol, adopt it in an ambiguous manner.

2. The Precautionary Principle marginalizes the role of scientists and can be applied in an arbitrary fashion (Chapman et al., 1998; Mahoney, 2000). This criticism is based upon the concern that the invocation of the principle usually involves the relaxation of the standards of proof normally required by the scientific community. In the face of evidence less rigorous than that required for "science-based" conclusions, decision making then invokes other, extra-scientific considerations.
3. The Precautionary Principle is used as a veiled form of trade protectionism. For example, it has been claimed that the "precautionary" decision by the European markets to ban American and Canadian beef (treated with growth hormone) had an element of protectionism (Adler, 2000; Foster et al., 2000). The essence of this criticism is that the principle is used to circumvent the fundamental rules established by trade agreements enforced by the World Trade Organization, which generally require a showing by an importing country of reliable scientific evidence that an exported product poses levels of risk not accepted in domestic products (e.g. the Sanitary and Phytosanitary Agreement adopted in the Uruguay Round of GATT). The Precautionary Principle, it is argued, inherently undermines the force of this requirement by taking the burden of scientific proof off the importing country and/or relaxing the rigour of the scientific evidence required to allege unacceptable risk. As alleged in Criticism 2, above, extra-scientific considerations then enter into a decision that should be "science-based".
1. The use of the Precautionary Principle is a form of over-regulation that will lead to a loss of potential benefits. For example, a strong biosafety protocol that limits the use of GE crops worldwide may retard advances in agricultural productivity, which could lead to global food shortages (Adler, 2000).

The persuasiveness of the latter three criticisms are clearly all related to the problem alluded to in the first one — the lack of uniform interpretation of the Precautionary Principle. The various interpretations of the principle cited in criticism (1), above, range over a wide spectrum, involving disagreement at several levels. These include disagreements over: 1) who should bear the burden of proof — those who allege potential harm, or those who deny it, 2) what the standard of proof should be for the party who bears the burden, and 3) to what extent the costs of precautionary restraint should be taken into account.

The most stringent (maximally precautionary) interpretations of the principle place the burden of proof upon the promoters of new technologies to prove its safety (no unacceptable risk), and require a high standard of proof that such risks are *not* involved. They counsel restraint, even if the social or economic costs of restraint are high. Proving "no-risk" in this sense is generally considered a difficult, if not impossible, scientific task.

The most permissive (minimally precautionary) interpretations of the principle, on the other hand, place most of the burden of proof upon those who allege potential risks, while perhaps relaxing the standards of proof (this is the only "precautionary" aspect), but they insist that the social and economic costs of exercising restraint be balanced against the potential risks. They "open the door to cost-benefit analysis and discretionary judgement" (Foster et al., 2000). The formulations of the Precautionary Principle in the Rio Declaration and the Cartagena Biosafety Protocol are both examples of this kind of cost-effectiveness approach.

In between these two extremes, are formulations of the principle that do not require proof of safety, but rather counsel restraint when levels of scientific uncertainty about potential risks remain high, with the burden of proof being assigned to those who develop or stand to benefit from the technology. These more moderate formulations, however, share with the more stringent formulations, the suspicion of permitting the prospect of significant benefits to override precautionary concern about the potential risks.

INTERPRETING THE PRINCIPLE

Although there is a wide diversity in the interpretation of the Precautionary Principle, it is possible to state its fundamental tenets, and to identify the points of debate within each of these tenets.

Recognition of Scientific Uncertainty and Fallibility

As noted above, the Precautionary Principle has its roots in a sense of scepticism about the ability of science, or any system of knowledge, to understand and predict fully the function of complex biological and ecological systems. The principle is essentially a rule about how to manage risks when one does not have fully reliable knowledge about the identity, character or magnitude of those risks. It assumes that there is often the possibility of error in the assessment of risks, and the higher the potential for this error, the greater the precaution it prescribes in proceeding with actions that place certain values at risk.

Uncertainty is an endemic and unavoidable aspect of any regulatory science, especially risk assessment science (Salter, 1988; Brunk et al., 1992). There are different kinds of uncertainty (Barrett, 1999) and many reasons for them. They include, among other things, the incompleteness and fallibility of the scientific models that are used to predict events and relationships in complex systems (Funtowicz and Ravetz, 1994), the incompleteness and inconsistency of data obtainable within the constraints of time and resources that normally operate within a regulatory context, and the presence of unavoidable but controversial extra-scientific assumptions (Brunk et al., 1992). The laboratory scientist can, and must, take the time and effort to reduce these uncertainties

before affirming or rejecting a scientific hypothesis. The regulatory scientist, however, often does not have the time or the resources to reduce this uncertainty.

The Precautionary Principle, however variously applied, is fundamentally a rule about how technology developers, regulators and users should handle these uncertainties when assessing and managing the associated risks. Having identified the potential for error in predicting all the outcomes, the rule identifies which of these outcomes it is most important to avoid (or protect) in the event that predictions turn out to be wrong. Is it best to have erroneously lost the potential benefits in order to avoid the potential harms, or to have erroneously suffered the harms in order to realize the benefits? The Precautionary rule tends to favour the former error.

One of the most commonly cited implications of the precautionary approach is the need to respect the distinction between "absence of evidence" and "evidence of absence" when assessing and managing technological risks. For example, the claim that "there are no known adverse health or environmental effects" associated with a particular technology can mean very different things. It can mean that rigorous and intensive scientific investigation of the potential harms that might be induced by the technology has failed to show any of those harms (and, in the best case, provided a reliable explanation why the harmful effects do not or will not occur). At the other extreme, this claim might mean simply that no studies to determine if the harmful effects occur have been carried out, in which case the claim is simply an admission of ignorance. In the first instance the claim would be "evidence of absence" (of risk); in the later instance it would be simply a veiled admission of the "absence of any evidence" relevant to the question. One simple expression of the Precautionary Principle is that it counsels restraint in proceeding with the deployment of a technology in the "absence of evidence", and requires that the greater the potential risks, the stronger and more reliable be the "evidence of their absence".

Presumption in Favour of Health and Environmental Values

The Precautionary Principle is a rule about handling uncertainty in the assessment and management of risk, and the rule recommends that the uncertainty be handled in favour of certain values — health and environmental safety — over others. Uncertainty in science produces the possibility of error in the prediction of risks and benefits. The Precautionary Principle makes the assumption that if our best predictions turn out to be in error it is better to err on the side of safety. That is to say, all other things being equal, it is better to have forgone important benefits of a technology by wrongly predicting risks of harm to health or the environment than to have experienced those serious harms by wrongly failing to predict them.

Understood in terms more familiar to scientists, the Precautionary Principle can be understood to require in general that, if an error in scientific prediction should occur, it is better that it erroneously predict an adverse effect where there is in fact none (false positive, or "Type I

error”), than that it erroneously predict no such effect when in fact there is one (false negative, or “Type II error”) (Shrader-Frachette, 1991; Barrett, 1999). The standards of scientific research are often understood to require just the opposite value judgment — that it is far more grievous for a scientist to commit the Type I than the Type II error. The Type I error involves making a premature claim (rejection of the null hypothesis — e.g. that a GM food poses no significantly greater risk) without ample scientific evidence. Committing the Type II error merely reflects a scientifically perspicacious withholding of judgment in the face of incomplete evidence. This is what makes the Precautionary Principle appear “anti-scientific” (Criticism 2, above) to many scientists. It would appear to ask regulatory scientists to risk committing the unscientific error of affirming risks that turn out to be much lower or non-existent (rejecting the null hypothesis when it turns out to be true).[*]

The rules of evidence in courts of law reflect a preference with respect to uncertainty analogous to that of science. In modern democratic societies, criminal courts favour the Type II over the Type I error. It is considered far worse to convict erroneously an innocent person of a crime than to acquit erroneously a guilty person. “Better that 10 guilty persons go unpunished than that 1 innocent person be convicted” is the well-known legal axiom. In the face of legal uncertainty (“reasonable doubt” in law), the presumption should be in favour of the null hypothesis (“not guilty”).

Thus, the Precautionary Principle appears to violate the rules of presumption that govern both scientific research and criminal law. Its acceptance in the regulatory context involves the judgment that, when it comes to regulating technological risks, it is better to err on the side of wrongly assuming risk than of wrongly assuming safety. This is the basis of Criticism 4 (above) that the Precautionary Principle tends to restrict the development of new technologies, and thus to retard the enjoyment of the benefits they may promise. It prefers to avoid risks, even at the expense of lost benefits, than to take those risks in order to enjoy the benefits. This, indeed, is the central force of the tenet — that given the potential of *at least certain kinds and magnitudes of*

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[*]While many biologists focus on avoiding the Type I error (e.g. set at 5%), and ignore the probability of Type II error, this is a weak application of statistical method. Many refereed ecological journals now demand that researchers calculate the power (1-β) of the statistical tests performed in any given experiment. There are many statistical resources readily available for analyzing power in almost any experimental context, and many biologists have advocated abandoning slavish devotion to avoiding the Type I error and paying much more attention to avoiding the Type II error, especially in applied contexts like resource management and conservation. In focusing on the Type II error the Precautionary Principle is, therefore, fully in accord with the current application of statistics in science. It does not, as the critics often charge, necessarily ask regulatory scientists to risk committing the “unscientific error” of affirming unwarranted risk. The Expert Panel is indebted to one of the anonymous peer reviewers of this Report for making this important point.

harms, reasonable prudence would slow the development of technologies pending stronger assurances of their safety or the implementation of active measures to guarantee safety.

The Precautionary Principle, however, need establish only a *presumption* in favour of safety over the benefits of a technology. Only the most stringent interpretations of the principle would demand that avoidance of risk, no matter how slight, always take priority over the enjoyment of benefits, no matter how great. Most interpretations of the principle (Pearce, 1994; Barrett, 1999) build in some sort of "proportionality rule" (O'Riordin and Jordan, 1995), which takes into consideration the costs of exercising precaution. The greater the opportunity costs of precaution, the more significant the potential harms and the more demanding the standards of evidence for suspecting such harms. Most proponents of the Precautionary Principle hold that the presumption in favour of safety increases to the extent that the potential harm to health and environment have characteristics such as irreversibility, irremediability or catastrophic proportions. It decreases to the extent that the harms are reversible and less probable, and the costs of precaution become excessively high.

As stated earlier, the most permissive (least precautionary) interpretations of the principle hold that the costs of exercising precaution should always be balanced against the risks — that is, that a simple risk–cost–benefit analysis should determine the levels of precaution. Such an approach would in effect negate the central point of the principle, which is to create a presumption in favour of safety, since it would insist that risks and benefits be given equal weight. Even more importantly, a pure risk–cost–benefit approach is seen by many critics as anti-precautionary. This is because the usual methods by which it is carried out have a built-in bias in favour of technological benefits, which are immediate, highly predictable and quantifiable (otherwise, the technology would have no market), and against the risk factors, which are discounted because they tend to be long term, less certain and less easily quantified (Shrader-Frechette, 1991).

Proactive Versus Reactive Approaches to Health and Environmental Values

Another common feature of appeals to the Precautionary Principle is inherent in the concept of "precaution" itself. It involves a requirement that the measures one takes in the face of potential harms are proactive rather than reactive. It makes the assumption that, with respect to certain kinds of technological risks, it is better to design and deploy the technologies in ways that prevent or avoid the potential harms, or guarantees the management of these risks within limits of acceptability, than to move ahead with them on the assumption that unanticipated harms can be ameliorated with future revisions or technological "fixes".

This proactive aspect of precaution entails certain norms for the development of technology, which include the responsibilities: a) to carry out the appropriate research necessary to identify potential unacceptable risks; b) to withhold deployment of technologies until levels of

uncertainty respecting these risks are reduced, and reasonable confidence levels concerning acceptable levels of risk are achieved; and c) to design technologies in ways that minimize health and environmental risks.

Burden of Proof and Standards of Evidence

In most legal proceedings, the party that alleges harm or offence on the part of another must shoulder the burden of proof that such harm has occurred and that it has been caused by the accused. In the case of criminal allegations, the prosecution has the burden of proof, and the standard of proof it must meet is that the evidence must establish guilt "beyond all reasonable doubt". In civil litigation, the plaintiff has the burden of proof, but the standard of proof the plaintiff must meet is usually less demanding — there must be merely a "balance of evidence" in support of the plaintiff's allegations.

Technology proponents often argue that the legal regulation of risk should follow similar principles — a technology, too, should be considered safe until proven unsafe (Miller and Conko, 2000). If the proof of risk is to be science-based in the strongest sense, it would follow that the standards of evidence should be those of research science — normally defined in terms of a 95% confidence rule (probability of error is less than 5%). This standard of evidence is the analogue in science to the "beyond all reasonable doubt" standard of evidence in criminal law.

The Precautionary Principle challenges the assumption that the regulation of environmental and health risks should always follow the legal analogy by asking whether such an approach constitutes an irresponsible attitude toward these risks. It is reasonable to invoke the legal analogy in regulatory science only on the assumption that any and all significant risks of this type can be predicted with high confidence by scientific research, not only in theory, but in actual regulatory practice. And, of course, invoking the legal analogue in regulatory science creates a strong presumption in favour of technological benefits rather than health and environmental safety. To paraphrase the legal axiom, it implies that "it is better that 10 hazardous technologies be employed to the detriment of human and environmental health than that one safe technology be erroneously restricted".

Consequently, the invocation of the Precautionary Principle nearly always involves an appeal either to shift at least some of the burden of proof (that the technology is *safe*) to those who propose the technology, or to relax in some way the standards of evidence required for the suspicion of unacceptable risk. Often it involves an appeal for both. Critics of the principle often argue that it puts the burden of proof upon promoters of a technology to *prove* (with low margins of error) its safety, which is simply unrealistic given the scientific impossibility of proving no risk (one can reject the null hypothesis, but not *prove* it using a standard statistical framework). There is no need to interpret the principle in such a manner, however. Proponents of the principle argue

that it is equally unreasonable to place the burden of proof upon the claim of unacceptable risk, especially if the standard of proof is the normal high confidence rule required by research science. The uncertainties endemic in regulatory science are too great for this burden to be met. Such a requirement would imply that, in a case where the weight of evidence suggested the possibility of serious risk to human, animal or environmental health but confidence in the data was substantially less than the rigorous levels required for laboratory science, there would be insufficient basis for regulatory restriction of the technology.

The Precautionary Principle can be interpreted in a manner that avoids both these extremes. It can be understood to place at least a fair share of the burden of proof upon technology proponents to show that the technology will not cause unacceptable risks to health or the environment — with standards of evidence something less than the highest levels of confidence in the conclusion of "no harm". Some proponents suggest that a better standard is the one analogous to that used in civil law — "balance of evidence". A "balance of evidence" standard, in conjunction with a burden of proof to the promoter of a technology, would mean that the promoter (i.e. the applicant for registration) would have the burden of establishing that at least the weight of evidence does not support a *prima facie* case of serious risk. Such an approach is much more precautionary than giving the burden of higher standards of proof to the side that alleges serious risk. But, it can be argued that it still is too lenient, since it permits the approval of technologies where there is substantial, though not *preponderant*, evidence that unacceptable risk exists. A more precautionary approach would invoke the simple maxim that the more serious the magnitude and nature of the potential harm to health or environment, the less demanding should be the levels of confidence (the wider the margin for error) in the assumption of risk.

If there are scientific data (even though incomplete, contested, or preliminary) — plausible scientific hypotheses or models (even though contested) — together with significant levels of uncertainty, that establish a reasonable *prima facie* case for the *possibility* of serious harm (with respect to reversibility, remediation, spatial and temporal scale, complexity and connectivity), then precautionary action is justified (Barrett, 1999; Tickner, 1999). "Precaution", as noted, does not mean paralysis; it means shifting the burden of narrowing the uncertainty range and removing the theoretical unknowns to those who wish to move forward with the technology.

Sometimes, a *prima facie* case of risk is established by preliminary evidence that is discounted by the scientific community. The British crisis over the link between BSE ("mad cow disease") and the human nvCJD (new variant Creuzfeld-Jacob Disease) provides an instructive example of precisely this situation. The Report of the British BSE Inquiry (BSE Inquiry, 2000) documents the manner in which the scientists (The Southwood Working Party) advising the British Ministry of Agriculture, Fisheries and Food (MAFF) assessed the preliminary evidence that BSE posed a health risk to humans. The Southwood Report assessed the risk to humans as

“remote”, but nevertheless made two recommendations it considered “precautionary” — that sick cows be taken out of the food chain and that bovine offal not be used in baby food. They did not recommend any further precautionary restriction on food use of subclinically infected animals (even though the long incubation period of BSE was well known). Because of the “remoteness” of the risk, such action was not considered “reasonably practical” (BSE Inquiry, 2000, Chapter 4).[1] The BSE Inquiry Report concluded that the scientific working group’s dismissal of the human health risks as “remote” was a significant factor in communicating to the government and to consumers that further precautionary measures were unnecessary. The Inquiry Report wondered why, if it was “reasonably practical” to be precautionary with respect to baby food, it is not also reasonable with respect to adult food, especially since the scientists had concluded their report with the caution that “if our assessment of these likelihoods are [sic] incorrect, the implications would be extremely serious.” Unfortunately, this caution was lost sight of by scientists and regulators, and was cited “as if it demonstrated as a matter of scientific certainty, rather than provisional opinion, that any risk to humans from BSE was remote” (BSE Inquiry, 2000).

What disturbed the BSE Inquiry most was the way the British MAFF responded to the preliminary assessment of the scientific work group. The Inquiry concluded that, rather than acting in an appropriately precautionary way, by taking steps to protect the British public against the potential “extremely serious” risks, the government became “preoccupied with preventing an alarmist over-reaction to BSE because it believed that the risk was remote.... The possibility of a risk to humans was not communicated to the public or to those whose job it was to implement and enforce the precautionary measures” (BSE Inquiry, 2000, Executive Summary). The implications of the BSE Inquiry Report are, therefore, clear: even when the available scientific evidence fails to establish a risk as anything other than “remote”, where there is a *prima facie* case of serious risk, significant (in this case highly costly) precautionary action is warranted.

Because the British government did not act early enough upon the growing evidence of human health risks, public confidence in both government and science was seriously eroded. As the Inquiry Report put it, “The public felt that they had been betrayed. Confidence in government pronouncements about risk was a further casualty of BSE” (BSE Inquiry, 2000, Executive Summary). The current moratorium on GM crops in the UK is widely seen as the only politically viable response to a public that has lost confidence in the ability of science, government or industry to protect public health.

Standards of Acceptable Risk (Safety)

Finally, the Precautionary Principle involves certain assumptions about what standards of safety are appropriately applied by risk regulators to different kinds of risk. The question of whether a technology is "safe" is widely recognized as a value judgment about whether a risk exceeds some level of acceptability. The acceptability of any given risk is determined by multiple factors, among the most important of which are the degree of voluntary choice involved in the risk taking, the off-setting benefits of the risk taking (and the fair distribution of the risks and benefits), the familiarity of the risk and the perceived ability to control it, the trustworthiness of the risk manager, and a whole range of highly subjective attitudes and fears associated with particular groups in particular circumstances (Fischhoff et al., 1981).

It is well known that risks associated with potentially catastrophic events (i.e. events involving dreaded harms occurring at high orders of magnitude, which are unforeseen and/or uncontrollable, and which may be irremediable) have extremely low levels of acceptability in public consciousness. When hazard magnitudes are catastrophic in nature, even extremely low probabilities of occurrence are often not sufficient to render the risk acceptable. These are the scenarios that typically invoke public demands for "zero-risk".[2]

Other safety standards commonly invoked in the context of health risks in food (e.g. chemical residues, microbiological risks, artificial additives) include "threshold" standards (those that set levels of acceptability at certain specified limits) such as NOAEL ("No Observable Adverse Effect Level) and "No Higher than Background Levels". In cases where risks and benefits tend to be evenly distributed among risk stakeholders (those who bear the risks also enjoy the benefits), so-called "balancing" standards such as risk–cost–benefit and cost-effectiveness standards tend to be more appropriate.

In Chapter 7, we identified a critical ambivalence in the concept of "substantial equivalence" as it is invoked in the regulatory environment of many countries and in international standards. We have expressed serious concerns about its use as a decision threshold for exempting new genetically engineered products from rigorous safety assessment, which, as noted above, may not always be consistent with a duly precautionary approach.

However, the concept also often serves a different function — that of establishing a standard by which a GM product can be considered safe for human and animal health and for the environment. Used in this way, it functions primarily as a "No Higher than Background Level" threshold safety standard. It sets a benchmark of risk acceptability, requiring that the health and environmental risks of GM products be no higher than those associated with their non-GM counterparts. It is based upon the assumption, not that traditional native and hybridized plants are entirely free of risks, but that whatever these risks may be, they are part of the normal background of risk that society has come to view as acceptable. If the employment of a new, GM food can be

shown (not assumed) to be "substantially equivalent" in the types and magnitudes of health or environmental risks to those posed by the employment of its traditional, non-GM alternative, by this standard it, too, should be considered acceptable or "safe".

Understood and applied in this way, "substantial equivalence" would appear to be a fairly rigorous precautionary safety standard. Consistently applied, it would question the safety of any GM food for which there was evidence of risks higher than those known to be posed by its traditional counterpart. It represents a more precautionary standard than the "balancing" standards (e.g. ALARA, Cost-Effectiveness, Risk–Cost–Benefit) typically employed by risk managers and regulators. These latter standards are all willing to "trade off" significant risks in order to limit the costs of safety or to realize certain economic and other benefits.

IMPLICATIONS FOR THE REGULATION OF FOOD BIOTECHNOLOGY

The debate over the meaning and proper application of the Precautionary Principle cannot be settled by this Expert Panel. However, because the principle has become deeply embedded in the many international agreements and protocols to which the Canadian government is a party, and is increasingly affirmed by European, North American and international regulatory bodies as a guiding principle for policy (CFIA, 1997; Barrett, 1999), it is appropriate that Canadian biotechnology regulatory policy reflect the basic sentiments and spirit of the principle. The recommendations contained in this Report assume that the fundamental tenets of the Precautionary Principle should be respected in the management of the risks associated with food biotechnology. All of these recommendations can be implemented within the existing regulatory framework. Our approach to the issues we consider within this Report is based upon what we consider the following precautionary rules:

RECOMMENDATIONS

8.1 In general, those who are responsible for the regulation of new technologies should not presume its safety unless there is a reliable scientific basis for considering it safe. This approach is especially appropriate for those who are responsible for the protection of health and the environment on behalf of the Canadian people. Any regulatory mechanism which assumes that a new product is safe on less than fully scientifically substantiated basis violates this fundamental tenet of precaution. The Expert Panel rejected the use of "substantial equivalence" as a decision threshold to exempt new GM products from rigorous safety assessments on the basis of superficial similarities (Chapter 7), because such a regulatory procedure is not a precautionary assignment of the burden of proof.

8.2 The proponents and developers of food biotechnology products bear a serious responsibility to subject these products to the most rigorous scientific risk assessment. In this sense, the primary burden of proof is upon those who would deploy these food biotechnology products to carry out the full range of tests necessary to demonstrate reliably that they do not pose unacceptable risks. The laws and regulations under which these products are regulated and approved in Canada already place this burden or proof upon producers of these technologies insofar as they require the producers or proponents to carry out the tests and submit data from these tests demonstrating that the products are safe.

8.3 Where there are scientifically reasonable theoretical or empirical grounds establishing a *prima facie* case for the possibility of serious harms to human health, animal health or the environment, the fact that the best available test data are unable to establish with high confidence the existence or level of the risk should not be taken as a reason for withholding regulatory restraint on the product. In such cases, regulators should impose upon applicants for approval of the technology the obligation to carry out further research which can establish on reasonable weight of evidence that unacceptable levels of risk are not imposed by the technology.

8.4 Serious risks to human health, such as the potential for allergens in genetically engineered foods, risks of extensive, irremediable disruptions to the natural ecosystems through emergence of highly aggressive or invasive weed species, or of serious diminution of biodiversity, demand that the best scientific methods be employed to reduce the uncertainties with respect to these risks. Approval of products with these potentially serious risks should await the reduction of scientific uncertainty to minimum levels. The Expert Panel supports the view of the British BSE Inquiry, as discussed above, in this regard. Even though the risks appeared remote on the basis of

the available evidence, the potential seriousness of the health risks justified extraordinary precaution before a fuller scientific picture was available.

8.5 Regulatory action in accord with the Precautionary Principle means the imposition of more "conservative" safety standards with respect to certain kinds of risks. Where there are health or environmental risks involving catastrophe scenarios (e.g. the potential effects of global warming), the greater the case for more conservative safety standards such as "zero-risk" or low threshold standards, such as that of "substantial equivalence", as articulated above. In the Panel's view, when "substantial equivalence" is invoked as an unambiguous safety standard (and not as a decision threshold for risk assessment) it stipulates a reasonably conservative standard of safety consistent with a precautionary approach to the regulation of risks associated with GM foods.

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NOTES

1. The Working Group invoked the principle known as ALARP (As Low As Reasonably Practicable). It requires an exercise in proportionality. When deciding whether a precaution is "reasonably practicable", it is necessary to weigh the cost and consequences of introducing the precaution against the risk which the precaution is intended to obviate.
2. The demand for "zero risk" is often viewed by risk experts as irrational, because there is no such thing as an absolute zero risk for any *possible* hazard occurrence. This is, strictly speaking, true. However, the demand for "zero risk" often can be interpreted as an expression of zero tolerance for any incremental *increase* in the already occurring background risk. For example, in the current debate about the impact of pollen from *Bt*-engineered crops upon the Monarch butterfly, there is evidence that these crops may pose some risk to the Monarch. But many argue that the risk is marginal in comparison with other greater risks imposed upon the species, such as destruction of its habitat. The question here is what level of risk is acceptably imposed upon this species. The insistence by some that *no* risk from *Bt* crops to the Monarch is acceptable is not a call for "zero risk" in any absolute form, but rather a call for zero *increase* in the cumulative risk burden already imposed upon the species.

9. ISSUES IN THE SCIENCE-BASED REGULATION OF BIOTECHNOLOGY

PART 1: MAINTAINING THE INTEGRITY OF RISK ASSESSMENT SCIENCE

One of the concerns frequently voiced about the regulation of biotechnology in Canada and elsewhere involves the question of the independence, objectivity and transparency of the science involved in the assessment of the technologies. This issue was raised as a concern by many of the parties who made submissions to the Expert Panel. It is generally framed in terms of public trust in the objectivity and disinterestedness of the scientists who develop, test and regulate biotechnology products. But it also concerns the process by which the underlying science used to assess GM products is made transparent to independent validation.

Trust in those who develop and regulate technologies is a factor in public acceptance of these technologies and of the risks they may involve. Studies of risk perception are uniform in the finding that even the most minimal risks may be unacceptable if levels of trust in those who manage those risks are low or eroding (Slovik, 1992; Powell and Leiss, 1997). Most commentators agree that the high levels of public apprehension in Europe about food risks generally, and GM food risks specifically, are significantly coloured by the loss of trust in scientists and regulators resulting from the BSE crisis in Britain (cf. Chapter 8). This is only one of the most dramatic examples of what numerous commentators have identified as a general erosion of public confidence in science well beyond Europe (Angell, 1996).

International trade protocols as well as national regulatory practices rely upon the ostensible objectivity and reliability of science in the assessment and management of risks associated with food biotechnology. Practices that compromise this objectivity and reliability also seriously erode public confidence in the regulatory process. Thus, the Expert Panel wishes to underscore the critical importance of this for the regulation of food biotechnology in Canada, and call attention to practices and social trends that tend, in fact, to compromise the scientific assessment of biotechnology risks.

Regulatory Conflict of Interest

One of the broadest issues relates to government regulators and policy makers. Biotechnology is viewed by most Western governments as an important part of the new economy. Many governments, including the Canadian government, have formal programs specifically designed to facilitate the growth of biotechnology (e.g. Alberta Science and Research Authority, 1996; Barrett, 1999). Many statements by Agriculture and Agri-Food Canada and Canadian Food

Inspection Agency (CFIA) officials and documents indicate that the official policy of the government regarding the regulation of agricultural biotechnology is two-fold — both the protection of the public from potential health and environmental hazards, and the ensuring of a viable and internationally competitive biotechnology industry (NBAC, 1987-88). As one Agriculture Canada official put it, the goal of regulatory agencies with respect to biotechnology must be to develop regulations that assure "that the products can be used without adversely affecting humans and animal health, and the environment", and that are "not so restrictive or time-consuming to fulfill that industry loses its competitive advantage and seeks markets outside the country" (Hollebone, 1988). CFIA has engaged in active media campaigns promoting agricultural biotechnology, and seeking to allay public fears about risks associated with GM foods (CFIA, 2000).

If the same government agency that is charged with the responsibility to protect the public health and environmental safety from risks posed by technologies also is charged with the promotion of that same technology, and if its safety assessments are, by official policy, balanced against the economic interests of the industries that develop them, this represents, from the point of view of both the public and the industrial stakeholders, a significant conflict of interest. Each stakeholder is placed in the position of having to ask, with respect to each regulatory decision, whether its own interests have been unduly compromised by the interests of the other.

The concern of the Expert Panel in this issue is not primarily from the point of view of the legal or ethical issues it raises. These are vitally important, but beyond the scope of the Panel's mandate. The Panel's interest is primarily from the point of view of how such regulatory conflict of interest compromises the integrity of regulatory science and decision making, as well as public perception of that integrity. The claim that the assessment of biotechnology risks is "science based" is only as valid as the independence, objectivity and quality of the science employed. All the regulatory departments involved in the regulation of food biotechnology should seek to separate institutionally as much as possible the role of promoter from the role of regulator. The more the regulatory agencies are, or are perceived to be, promoters of the technology the more they undermine public trust in their ability to regulate the technology in the public interest.

Confidentiality Versus Transparency in Canadian Regulatory Science

Current regulatory practice in Canada protects the confidentiality of much of the test data submitted by developers of food biotechnology in support of the approval of their products. Data identified by such companies as Confidential Business Information (CBI) is protected under federal access to information laws. This information can be released only by application, and with approval of the owner of the proprietary information. This means that the full data in the risk assessments upon which approval (or non-approval) decisions are based are often not available for

public scrutiny or for peer review in the community of science. The company applying for approval of a biotechnology product essentially gets to decide what counts as CBI. Presumably, the regulatory agency can, and often does, negotiate with the company applicant what test data the agency will consider confidential, and thus has the power to negotiate for relatively full disclosure.

The information that CFIA makes available to the public, contained in published Decision Documents, summarizes the assessment conclusions upon which the approval of the unconfined release of a genetically engineered plant into the environment was based. The actual data and scientific judgments leading to that assessment are not included in the Decision Document. Thus, the science behind the regulatory decision remains largely obscure unless there is an application to view it made under access to information laws. While one could make the argument that some of the data provided to regulators need to be protected (e.g. those related to genetic transformations and gene constructs), the Panel does not agree that data pertaining to environmental and ecological consequences should be proprietary.

It is important to note, however, that the amount of information the regulatory departments choose to disclose from the application and approval process is not set by any formal regulations. Rather, it is a policy judgment that seeks to balance the interests of industry against the desire for transparency in the regulatory process. Government could insist on more complete disclosure of the relevant data, but many consider that such a policy discourages industry research and development. In the extreme case, a company may decide not to seek approval if it fears that the application process would lead to the disclosure of valuable business information.

In meetings with senior managers from the various Canadian regulatory departments, the Expert Panel addressed questions related to their handling of the issues of transparency and confidentiality in dealing with applicants for licensing of new biotechnology. Their responses uniformly stressed the importance of maintaining a favourable climate for the biotechnology industry to develop new products and submit them for approval on the Canadian market. If the regulatory agencies do not respect industry interests in protecting the confidentiality of product information as well as data obtained from extensive health and environmental testing, industry in turn will be deterred from engaging in the regulatory approval process. Several of the managers referred to the importance of maintaining a relationship of trust between industry and the regulators. Only in an atmosphere of trust, they argued, can government and industry work together in the cooperative way necessary to generate the product and test data required for the protection of public safety.

Such concern with industry development, though understandable, highlights another aspect of the regulatory conflict. The conflict of interest involved in both promoting and regulating an industry or technology, discussed in the previous section, is also a factor in the issue of

maintaining the transparency, and therefore the scientific integrity, of the regulatory process. In effect, the public interest in a regulatory system that is "science based" — that meets scientific standards of objectivity, a major aspect of which is full openness to scientific peer review — is significantly compromised when that openness is negotiated away by regulators in exchange for cordial and supportive relationships with the industries being regulated.

In the judgment of the Expert Panel, the more regulatory agencies limit free access to the data upon which their decisions are based, the more compromised becomes the claim that the regulatory process is "science based". This is due to a simple but well-understood requirement of the scientific method itself — that it be an open, completely transparent enterprise in which any and all aspects of scientific research are open to full review by scientific peers (Kennedy, 2000). Peer review and independent corroboration of research findings are axioms of the scientific method, and part of the very meaning of the objectivity and neutrality of science.

Validation of the Science

In principle, the Regulations specified by CFIA, Food and Drugs Act, and Canadian Environment Protection Act for approval of GMOs, particularly those that pertain to microbes and plants, are comprehensive in their breadth of required information, ranging from the molecular nature of the novel gene construct to potential consequences to human health and the environment. However, despite this breadth, the Panel has concluded that there is no means of determining the extent to which these information requirements are actually met during the approval process, or of assessing the degree to which the approvals are founded on scientifically rigorous information. The Panel attributes this uncertainty to a lack of transparency in the process by which GMOs are approved within the present regulatory framework.

The Panel's, and the public's, lack of access to this information raises questions concerning the scientific rigor of the approval process. Based on the Guidelines that accompany the CEPA and FDA Regulations, and based on interviews with representatives of CFIA, Health Canada and Environment Canada, the Panel concluded that, although the proponents are required to provide new data in some areas, there is no means for independent evaluation of either the quality of the data or the statistical validity of the experimental design used to collect those data. Furthermore, it appears that a significant part of the decision-making process can be based on literature reviews alone.

Consider, for example, the sole Regulation under CEPA that deals with the potential risks of non-microbial transgenic organisms to the environment. Schedule XIX (Sections 29.16 and 29.19), paragraph 5c identifies the requirement for information on "the potential of the organism to have adverse environmental impacts that could affect the conservation and sustainable use of

biological diversity." The information necessary to meet the requirement stipulated by this Regulation is detailed in CEPA Guideline 4.3.5.3, which states:

> "A brief summary of predicted ecological effects should be provided, including any effects on biodiversity. This should include a description of the expected beneficial or adverse ecological effects that result from the growth of the organism, as well as any other ecological effects likely to occur from its introduction."

The Panel interprets this Guideline to mean that the CEPA Regulation pertaining to environmental risks associated with non-microbial transgenic organisms has no explicit data requirements for information pertaining to the potential effects of these GMOs on conservation and biodiversity. (This may reflect the fact that Regulations have yet to be developed for transgenic animals by CFIA and for transgenic fish by Department of Fisheries and Oceans.) It is the Panel's opinion that a literature review alone is insufficient and that experimental data for the particular GMO under consideration should be part of the evaluation process.

Currently, there is no objective way for the public or independent scientists to evaluate fully the scientific rigor of these assessments. In the one example available to the Panel, the data used to evaluate the invasiveness of Monsanto's *Roundup Ready* Canola (approved in 1995) were judged by Barrett (1999) to be scientifically inadequate for either a rational regulatory decision-making process or a peer-reviewed scientific publication. Based on available information, this is a judgment with which the Panel agrees. However, the generality of this conclusion cannot be assessed because all of the data sets used in the decision-making process, notably those pertaining to environmental safety, are not available for public scrutiny.

The Panel concludes that the lack of transparency in the current approval process, leading as it does to an inability to evaluate the scientific rigor of the assessment process, seriously compromises the confidence that society can place in the current regulatory framework used to assess potential risks to human, animal and environmental safety posed by GMOs.

Increasing Commercialization of University Scientific Research in Biotechnology

There is growing concern in the public and the scientific community that the increasing focus of government upon the promotion of biotechnology has an adverse impact on the allocation of research funds. As suggested by Varma, there is a growing perception that "Basic science is valued only if it contributes to the creation of products or processes for... industry. The government agencies are more and more supporting research which is geared to help industry" (Varma, 1999). In Canada, an Expert Panel on Commercialization of University Research has recently made strong recommendations to the Prime Minister's Advisory Council on Science and Technology that governments and universities adopt policies encouraging the commercialization

of university research with intellectual property potential (Expert Panel on Commercialization, 1999).

There are also numerous specific conflicts which have been associated with the research environment. Though academic science has always been affiliated with the private sector, the application of genetic engineering to food production is progressing at a time when universities and university researchers are building unprecedented ties with industry partners (Schultz, 1996; Angell, 2000; DeAngelis, 2000). Researchers, such as David Blumenthal, have noted that these commercial alliances can have a profound impact on the choice of research topics (Blumenthal, 1992). They also help to create an atmosphere of secrecy among researchers (Wadman, 1996; Blumenthal, 1997; Caulfield, 1998; Gold, 1999) and jeopardize the trust which the public places in academic science. As noted by Korn: "There is good reason for concern [that the] idealistic image of academic virtue and the public's willingness to trust in it may be tottering" (Korn, 2000).

The pressures and opportunities for institutional and personal gain from research has a profound impact upon the willingness of researchers to share openly research plans, research results and relevant resources within the research community. This openness is one of the traditional strengths of the scientific enterprise. It is the traditional mechanism by which the potential risks and failures of certain technological designs and directions become widely known within the scientific and technological communities. This is true not just of biotechnology, but of other research disciplines with potential industrial applications as well. Increased secrecy and protection of intellectual property in the research community does not well serve the public interest in reliable scientific research on safety matters.

Academic/industry relationships are extremely widespread. Blumenthal's 1997 study found 90% of the US life sciences companies surveyed had a "relationship with academia". In such a climate, it may become increasingly difficult to find independent academic researchers with the motivation, or even the freedom, to evaluate the claims of industry. As argued by science historian Charles Weiner: "[T]he dual roles played by many leading biologists have begun to impair the credibility of scientists when they provide advice on matters of public concern relating to their research" (Weiner, 1988, at 32–33). Scientists who concentrate their research efforts on the environmental and health risks of new technologies, and who develop the expertise upon which competent regulation of these technologies must depend, are not likely to be prime candidates for research grants from industry partners.

In addition, academic scientists involved in the advancement of knowledge in the biotechnology area are increasingly enticed by the considerable commercial value of this knowledge, and increasingly involved in the patenting and marketing of new organisms and techniques. This situation is exacerbated by the emerging structures of intellectual property ownership and management by public universities. A university researcher wishing to release the

results of his or her work in the interest of the public good may encounter tangible institutional or corporate pressure not to do so in order to capture the potential commercial value through patenting and licensing. In relation to food biotechnology, it is arguable that such a refocusing of the public research agenda makes it more difficult to find funds for research aimed at the critique or evaluation of GMO technology or scientific researchers with the independence and objectivity to carry it out.

This co-opting of biotechnology science by commercial interest contributes to the general erosion of public confidence in the objectivity and independence of the science behind the regulation of food biotechnology. It reduces significantly the scientific resources available to government regulators of the technology and, hence, the reliability of the "science base" of this regulation. This situation is one that goes well beyond the power of government regulatory agencies to remedy on their own. Instead, they suffer the consequences of these dynamics in the society insofar as the knowledge base they depend upon for the evaluation of technological risks is impoverished. The Expert Panel considers this to be a serious public policy issue related to the public funding of independent scientific research in the universities, and can be remedied only by those in government who formulate and implement these public policies.

RECOMMENDATIONS

9.1 The Panel recommends that Canadian regulatory agencies and officials exercise great care to maintain an objective and neutral stance with respect to the public debate about the risks and benefits of biotechnology in their public statements and interpretations of the regulatory process.

9.2 The Panel recommends that the Canadian regulatory agencies seek ways to increase the public transparency of the scientific data and the scientific rationales upon which their regulatory decisions are based.

9.3 The Panel recommends that the Canadian regulatory agencies implement a system of regular peer review of the risk assessments upon which the approvals of genetically engineered products are based. This peer review should be conducted by an external (non-governmental) and independent panel of experts. The data and the rationales upon which the risk assessment and the regulatory decision are based should be available to public review.

9.4 The Panel recommends that the Canadian Biotechnology Advisory Commission (CBAC) undertake a review of the problems related to the increasing domination of the public research agenda by private, commercial interests, and make recommendations for public policies that promote and protect fully independent research on the health and environmental risks of agricultural biotechnology.

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PART 2: LABELLING OF GENETICALLY MODIFIED FOODS

A major issue in the public debate over food biotechnology has concerned the labelling of foods containing, or produced from, GM products (Pollara, 2000). In part this issue has been cast as a human health issue — if GMO foods pose risks to health, the consumer should have the right of "informed choice" about exposure to these risks. However, it is also in significant part a socio-economic and political issue having to do with the alleged right of consumers to participate intelligently in the marketplace and to exercise the "power of the pocketbook" in support of the technologies and industries they prefer. Public opinion surveys (Leger and Leger poll, March 2000) report that consumers feel that they have not been sufficiently informed (67.7% of responses) to make educated decisions on the adoption of GM food.

Because the first generation of GM foods has been aimed largely at producing food industry benefits (e.g. increased yields, lower production costs), consumers have yet to perceive direct benefits to them from biotechnology in food production. This has contributed to the perception that GM plants benefit large corporations that bear few of the risks, while providing little or no benefit to consumers, who may bear the potential risks. The absence of labelling on GM products has reinforced the perception that companies are "hiding" important information from the public. The absence of justification for the need for GM food, combined with a perception of lack of transparency from regulatory agencies, and the absence of balanced risk/benefit analyses have all undermined the acceptance of these products.

The Expert Panel is compelled to address the labelling question because concerns about health and environmental risks form an important part of the arguments made in favour of various forms of labelling. As argued below, one of the functions of food labelling is to turn over certain risk management functions to the consumer — which Canada does currently with its labelling policies regarding known allergy risks or health-related nutritional changes. The question we address in this chapter is whether the genetic food technologies we have assessed in this Report involve potential hazards or risks whose effective management would require the use of food labels. If so, are those risks better addressed through the use of general *mandatory* labels (i.e. required labels for all GM foods and foods containing GM components) or *voluntary* labels (i.e. labels used voluntarily by producers to provide information that enhances the product for some consumers).

Current Labelling Policies on GM Foods

Many countries have introduced some form of mandatory labelling for GM foods (Nottingham, 1999). Mandatory labelling requirements have been implemented in the EU and are being implemented in Japan. The governments of Australia and New Zealand have agreed "in principle" that GM products should be labelled, including labelling that foods "may contain" GM

ingredients (CFIA, 2000). The US, like Canada, currently requires only those GM food products that pose health and safety issues such as possible allergens or changed nutritional content from accepted levels to be labelled. However, there is currently legislation introduced in both houses of Congress to require labelling of food that “contains a genetically engineered material, or was produced with a genetically engineered material” (Goldman, 2000).

The primary forum at the international level for discussion of the labelling issue is the Codex Alimentarius Commission. The Codex Committee on Food Labelling (CCFL) has been carrying on this debate for several years, without resolution. It is divided between those member nations that believe that mandatory labelling should be product-based only and those that believe it should be based on differences of process, such as the rDNA technologies (IFT Report, 2000). One international agreement, however, does speak to the GM labelling issue. The Cartagena Protocol on Biosafety has been interpreted to provide that “living modified organisms” (LMOs) intended for “food, feed or processing” must be identified as LMOs (IFT Report, 2000). The US Department of State has interpreted this provision to require only “may contain” labels on international shipments of LMOs, and not to impose consumer product labelling requirements (IFT Report, 2000).

To date, Canada has taken no formal steps to introduce a mandatory labelling scheme, although the government is currently in the process of developing regulations governing the labelling of biotechnology products (Wilson, 2000). The Canadian government is supporting a joint initiative with the Canadian General Standards Board and the Canadian Council of Grocery Distributors to develop a Canadian standard for voluntary labelling of GM products, similar to the recently adopted voluntary labelling standard for organic products (CFIA, 2000).

Public support for labelling appears, in many respects, to be rooted in a widely held belief in the value of informed choice and the “right-to-know”. Several of the letters received by the Panel from interested parties raised a commonly heard public argument: that GM foods involve unknown or uncertain risks, and that consumers are being used as “human guinea pigs” in a large experiment to determine what these might be. If consumers are subjects of this experiment, the argument continues, they at least should have the right to informed consent to participation, and this can be exercised only if they have appropriate information (i.e. food labels).

Labelling is also usually defended as an important mechanism of risk management, in which the decision whether or not to be exposed to potential hazards in a product is shifted to the consumers or endusers, as is the responsibility to manage those hazards as they choose. If done using meaningful information, it allows individual consumers to make choices about the acceptability of a given risk to themselves. Labels warning consumers to “cook properly before serving” are examples of this type of risk management. Rather than removing products from the market that may contain hazards such as *E. coli* or *Salmonella* contamination, notification of the

risk passes the effective management of that risk on to the consumer. Labels warning that a product contains ingredients that are known major allergens (e.g. peanuts) serve a similar purpose. Rather than removing the product, and thus also the risk, from the market entirely, the management of the risk is left to those who purchase and consume the product.

In Western regulatory jurisdictions, labelling has generally been thought to be mandated only when there is some feature of the *product* itself that is worthy of being brought to consumers' attention, such as a specific health risk or nutritional issue (CFIA, 2000). The *process* by which a food product is produced (e.g. by genetic modification) has generally been considered to be irrelevant.

In the US, the courts and the Food and Drug Administration (FDA) have generally considered it a requirement that a mandatory food label refer to a "material fact" about the product that is relevant to nutritional value or safety (IFT Report, 2000). In this regard, the issue is closely tied to the concept of substantial equivalence. If a food product is "substantially equivalent" to an existing product, it is assumed that no labelling is required. This philosophy toward labelling was consistently expressed to the Panel by representatives from the CFIA and Health Canada. In the context of GM foods, a new and identifiable health safety risk, such as the presence of a new allergen, or a substantial alteration in the nutritional properties, would need to exist in order to justify labelling under the current Canadian and US regimes (Miller, 1999; CFIA, 2000).

The recent response by many countries to GM food products, particularly in the EU, appears to be a departure from this general rule of product-based, health risk labelling. The decision to mandate labelling of these products in Europe is considered by its critics to be a political response to a broad range of public concerns rather than a reflection of scientific evidence calling into question the actual safety of GM foods. Others argue that it is the result of European governments having been more responsible about informing their citizens of the potential risks and their taking a more precautionary approach toward uncertain risks (Le Monde, 2000). Throughout the 1990s, there was growing pressure in the EU to introduce some form of labelling of all foods and ingredients produced by genetic engineering, regardless of whether they were demonstrably different from those derived from traditional, non-GM plants (Nottingham, 1999). This poses the question whether Canadian regulators should adopt a similar approach to labelling for some or all products associated with the genetic modification process.

In both Canada and the US, there is an important exception to the general rule that labelling should be product-based, which could be seen as a precedent for GM foods. Both countries have a mandatory labelling requirement for foods that have been subjected to the process of irradiation (Food and Drug Regulations, B.01.035; IFT Report, 2000). As with GM food products, there has always been a degree of public and consumer suspicion about the safety

of food irradiation — a process used to reduce the presence of pathogens in food products (Lutter, 1999). Though there are many agencies, including the World Trade Organization, that support the use of irradiation for food preservation (Nightingale, 1998; Lutter, 1999), it remains a relatively tightly regulated food preparation process. In the US, the labelling requirement is justified by defining the irradiation process itself as a "food additive" (Pauli, 1999). The rationale behind this regulatory approach is that irradiation is a process that "can render food materially different organoleptically, e.g. taste, smell and texture". Although the USFDA no longer considers this rationale to have any firm scientific basis, the labelling requirement has been maintained (IFT Report, 2000). However, even if it had such a basis, it is clear that these "material facts" about irradiated foods have no scientifically established relation to health or nutrition risks.

In Canada, the regulatory requirement for labelling of irradiated foods is laid out in Section B.01.035 of the Food and Drug Regulations. This regulation requires that both non-pre-packaged and pre-packaged foods carry a label stating that the food has been irradiated and carrying the international symbol for irradiation. Even pre-packaged foods containing more than 10% of irradiated ingredients must list every such ingredient on the label, preceded by the statement "irradiated" (Section B.01.035.6). Thus, the argument that there is no precedent for process-based labelling in Canada is not accurate. Nor is the claim of no precedent for the labelling of processed foods containing only a percentage of ingredients subjected to a specific process such as irradiation (or, presumably, genetic engineering). Indeed, it could be argued that the case for labelling of GM food products is stronger than for irradiated ones, because genetic engineering may produce "material changes" in the product itself. In the case of quality-enhanced products (e.g. improved appearance, longer shelf-life), this is the whole point of the genetic engineering.

Socio-Political and Ethical-Philosophical Concerns

As noted, the dominant argument for mandatory labelling of GM foods rests upon the claim that it enhances informed choice among consumers. Critics of biotechnology often point out that, while the biotechnology industry argues that the market should be allowed to decide whether GM food products are acceptable, it at the same time often opposes the very labelling necessary for consumers to exercise informed choice. In response, the opponents of labelling point out the myriad of complications involved in formulating a labelling policy that would actually provide accurate and meaningful information to consumers (see below), and conclude that labelling does not solve the problem.

The complicating factor in this debate is that, as the situation in the EU illustrates, the issues about which many consumers wish to exercise informed choice go well beyond the restricted range of health concerns. They involve a much broader range of religious/philosophical, ethical, social and political issues. These are summarized in Chapter 1. These broader dimensions

of the labelling debate are beyond the mandate of the Panel. We will, therefore, withhold comment on the question of whether mandatory (or voluntary) labelling would provide a feasible means of enhancing consumer choice with respect to these issues, or whether it would be a socially desirable means of achieving this goal. However, policy makers need to recognize that public demand for the labelling of GM products is not based solely on health considerations.

Health Basis for Mandatory Labelling

The Expert Panel is unanimous in its support for mandatory labelling of GM food products where there are clear, scientifically established health risks or significant nutritional changes posed by the product itself. The Panel sees several kinds of justification for the mandatory labelling of these products.

- The first justification stems from the fact that certain changes introduced into food products, regardless of the means by which they are introduced, pose clear, scientifically established risks only to some consumers and not others (e.g. pregnant women or persons with allergies to peanuts or fish). In such cases, there is a non-controversial case for relying upon the consumers themselves to manage these risks. In other words, providing the consumers with a clear warning label permits those at risk to protect themselves by not consuming the product, while at the same time permitting those who are not at risk to consume the product.
- The second justification rests upon the recognition that there are some kinds of hazards in food products that place all consumers of those products at risk to some degree, but the consumers have the right to decide for themselves whether, or at what levels, they wish to be exposed to the risk. In other words, risk management is transferred to consumers in order to allow them to determine their own levels of acceptable risk, rather than having these determined for them by regulatory standards. Warnings on tobacco and alcohol products are examples of this rationale for labelling.
- There is a third, more controversial, justification for food labelling that is rooted in the well-documented fact that consumer perceptions of the acceptability of certain risks are strongly related to the levels of uncertainty in the assessment of these risks. Risks fraught with high levels of uncertainty, but associated with catastrophic outcome scenarios or "dreaded" hazards (e.g. cancer in our society) are usually less acceptable (Slovic, 1991). Therefore, an argument can be made that in cases where there are recognized uncertainties in the identification or evaluation of certain risks, labels warning of the existence of these uncertainties are useful and prudent. They allow the consumer to decide whether the risks, however minimal, are acceptable. For example, labels stating that a food contains genes engineered into it from sources that are known to be allergenic (e.g. a peanut gene spliced

into soybean) may be advisable, even though there is no evidence that the known allergenic protein has in fact been transferred. A scientist would likely judge the risk in this case to be negligible, but a person with a lethal allergy to peanuts may have a legitimate interest in avoiding the product.

This last justification for mandatory labels on food products is much more controversial than the first two because it departs from the generally accepted principle that a label should communicate only firmly established health or nutritional information. Otherwise, it is often argued, the label does not provide guidance to consumers, but leaves them speculating about the significance of the information and filled with unanswered questions.

Conclusions on Mandatory Labelling

In assessing the justifications for labelling, the Panel has focused primarily on whether mandatory labelling on the basis of health and environmental risk is a policy that could be justified on the basis of a *scientific* assessment of these risks. We were concerned particularly with the question of whether, from a scientific point of view, there was sufficient reason to require mandatory labelling for GM foods, while not requiring it for novel and exotic foods produced by more traditional non-GM processes. The Panel also attempted as much as possible to distinguish the socio-political justifications from the health and safety considerations and to limit its consideration to the latter. The issue of whether a general mandatory labelling of GM products would be an effective instrument for managing the health and environmental risks uniquely associated with food biotechnology generated a great deal of controversy among the Panel members. In the end, however, the Panel concluded that there was not at this time sufficient scientific justification for a general mandatory labelling requirement. However, the Panel concluded that many of the concerns identified in this Report do call for a strongly supported voluntary labelling system for GM foods.

The Panel wishes to emphasize, however, that these conclusions are premised upon the assumption that the other recommendations of this Report concerning the conditions for the effective assessment and management of the risks of GM organisms are fully implemented by the regulatory agencies. If proper assessment and *long-term* monitoring procedures are carried out, and the appropriate safety standards enforced, then any significant health and environmental risks of GM organisms should be identifiable, and the products can be either disapproved or approved on the condition of explicit labels warning of the risk (e.g. allergens or nutritional deficiencies).

The Panel also wishes to emphasize that the issue of uncertain environmental impacts from GM organisms crosses over the somewhat fuzzy line between clearly established risk concerns, which are the Panel's sole mandate, and the broader socio-political concerns, which are commonly advanced in favour of mandatory labelling. Our conclusion with respect to mandatory labelling on

the basis of risk and safety concerns should not be read as prejudicing in any way the debate about labelling on these broader grounds.

If the testing procedures we recommend elsewhere in this Report disclose a new allergen, health risk or nutritional variation, labelling would, of course, be required. This approach would be consistent with the present regulatory approach. It is important to note, however, that while we believe that labelling should be reserved for specific health risks and nutritional variations, identifying which risks justify a label may not be easy (e.g. would the existence of a possible new allergen justify a label or should regulatory approval be withheld entirely?). Though such issues will require ongoing consideration by the relevant regulatory agencies, they do not alter the Panel's general recommendation that a general mandatory labelling scheme is not advisable.

One of the most persuasive considerations for many of the Panel members was that, given our current knowledge about the risks associated with GM foods compared with similar non-GM food products, we see little scientific reason for treating the two differently with respect to labelling requirements. There may be uncertain and currently unpredictable health and environmental risks associated with the long-term production and consumption of GM products. Indeed, other chapters of this Report have identified areas of such potential risks. However, there are also uncertainties and unknowns about the long-term health implications of many non-GM food products. To mandate labelling for potential health risks in GM products alone would promote an inconsistency with no firm scientific justification.

Voluntary Labelling

The preceding considerations have led the Panel to conclude that there are not currently sufficient reasons to adopt a system of general mandatory labelling of GM foods. They do not lead necessarily to the same conclusion about voluntary labelling. Many of the concerns voiced in favour of mandatory labelling can be addressed, at least in part, by voluntary labels. This is true, not only of the social, ethical and political concerns, but also of some of the risk-related concerns, especially those related to uncertainties and even fears about unsubstantiated risks associated with GM foods.

Elsewhere in this report the Expert Panel has identified what it considers to be the most significant risks to human, animal and environmental health posed by current and future food biotechnology products. Chapter 4 (Part 1) identified certain difficulties involved in using traditional toxicological models to identify and assess the health risks associated with GM food products, especially GM foods in their entirety. Chapter 4 (Part 2) also identified the difficulties related specifically to the identification and assessment of potential allergens in novel foods, and concluded that there are currently available no testing protocols that can reliably overcome all these difficulties. It can be expected that new allergic reactions will develop in populations as a

result of exposure to new proteins introduced into these foods, and it will not always be possible to predict these reactions. Chapters 5 and 6 have identified potential health and environmental risks posed by GM animals and plants while recognizing that the probabilities of their occurrence and the magnitudes of their harm are difficult to assess (e.g. the risks to aquatic environments of escaped GM fish). Even were these outcomes well established, in many cases there would be widespread disagreement about their acceptability (e.g. loss of habitats or of biodiversity).

The Panel does not believe that these identifiable but relatively uncertain risks are appropriately managed by means of a *general* mandatory labelling requirement. However, many consumers have strong interests in exercising the power of consumer choice in the market with respect to these environmental and health safety issues. The Panel believes that strong government support for voluntary labels is an effective way of providing consumer input into these issues, and encourages the Canadian regulatory agencies responsible to establish guidelines for the regulation of reliable, informative voluntary labels.

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GLOSSARY

abiotic	arising from non-biological sources
adjuvant	a preparation used to stimulate the immune response during antibody induction
agonistic behaviour	competitive behaviour
Agrobacterium tumefaciens	a bacterium used in the process of creating GM plants. In nature, a soil bacterium responsible for the “crown gall” disease in some plants
allele	one of two or more copies of a gene in plants or animals
allergen	a substance, usually a protein, capable of inducing a specific immune hypersensitivity response, often resulting in immunoglobulin E production
allergy	a hypersensitive state involving the immune system as a result of exposure to certain substances, usually foreign proteins. Food allergy (food hypersensitivity) is an abnormal immunologic reaction usually resulting from the ingestion or contact with a food or food component. This term often refers to immunoglobulin E-mediated mechanisms but may include any immune response to a food.
allometry	differential growth of body parts; change of shape or proportion with increase in size
anadromous	fish that return from oceans to fresh water to spawn (e.g. salmon)
anaphylaxis	an acute, severe, sometimes fatal allergic reaction affecting two or more body systems. It results from binding of immunoglobulin E to sensitized immune cells (mast cells and basophils), with release of chemical mediators that cause multiple adverse effects on target organs.
animal commodification	the treatment of animals as commodities rather than as beings with intrinsic worth

animal welfare	most widely used in the sense of encompassing the "Five Freedoms for Animal Welfare". First formulated by the Farm Animal Welfare Council, a body set up by the UK government, in response to the Agriculture (Miscellaneous Provisions) Act of 1968, to advise on issues relating to farm animal welfare and to develop new standards for agricultural practice. Their five freedoms define the needs of animals which should be met under all circumstances: freedom from hunger and thirst freedom from thermal and physical discomfort freedom from pain, injury and disease freedom from fear and stress freedom to express normal behaviour
antibiotic resistance markers	see selectable marker gene
antibody	a gamma globulin or immunoglobulin produced by the immune system in response to exposure to a specific substance, termed an antigen. Five major immunoglobulin classes exist in humans, IgG, IgM, IgA, IgD and IgE.
antifeedant	plant secondary metabolite that reduces or inhibits feeding by a herbivore. Most plants produce these compounds as a means of defence against natural enemies.
antigen	a substance, usually a high molecular weight protein, polysaccharide or complex, which is capable of inducing specific immune responses, including antibody formation
antinutrient	an undesirable substance in food
Arabidopsis	small plant of the mustard family commonly used to study plant genetics and plant genomics
assortative mating	mating of like phenotypes: resistant with resistant and susceptible with susceptible
atopy	a hereditary tendency to develop allergic diseases which include asthma, allergic rhinitis, food allergy and atopic dermatitis (eczema), in associating with a tendency to oversynthesize IgE antibodies
base pair	two bases that form a "rung of the DNA ladder". A DNA strand consists of a chain of nucleotides, each of which is made of a molecule of sugar, a molecule of phosphoric acid, and a molecule called a base. The four bases used in DNA (A,T, G and C) are the "letters" that spell out the genetic code (see DNA).

biodiversity — the number and types of organisms in a region or environment. Includes both species diversity and genetic diversity within species.

biological invasion — the introduction of an organism into a new environment or geographical region followed by rapid multiplication and spread

biotechnology — a set of biological techniques developed through basic research and now applied to research and product development. In particular, the use of recombinant DNA techniques.

biotic — from biological sources

broodstock — the group of males and females from which fish are bred for aquaculture

Bt, Bacillus thuringiensis — a soil bacterium that produces a toxin that is deadly to some insects. Many strains exist, each with great specificity as to the type of insects it can affect.

Canadian Nutrient File — a compilation of nutrient values for foods available in Canada, produced by Health Canada

carrying capacity — the maximum number of organisms of a given species that can be supported in a given area or habitat

catecholamines — neurotransmitters in mammals (e.g. adrenaline)

cellularity — characterizes the physical and chemical properties of cells found within a specific tissue

cellulolytic — the capacity to digest cellulose

chemoautotrophic — an organism capable of deriving its metabolic energy from mineral sources

chimera — an organism containing two or more genetically distinct cell or tissue types

chromatography — a technique for separating complex mixtures of chemicals or proteins into their various constituents

chromosome — one of the threadlike “packages” of genes and other DNA in the nucleus of a cell. Different kinds of organisms have different numbers of chromosomes.

clone — descendants produced vegetatively or by parthenogenesis (development of an ovum without fertilization) from a single plant, or asexually or by parthenogenesis from a single animal. More generally, organisms derived by division from a single cell.

confined field trial	field trial carried out with specific restrictions on location, plot size, etc.
conformational epitopes	epitopes whose form derives from specific transient folding patterns in a protein
congeneric	belonging to the same genus
conspecific	belonging to the same species
cross-compatible	the ability of two related organisms to exchange genes through sexual reproduction. Also referred to as inter-fertility.
cry	designation of a gene encoding insecticidal crystal proteins in the soil bacterium *Bacillus thuringiensis*
delta-endotoxins	*Bt* insecticidal proteins
developmental asynchrony	a pattern of development within sub-populations that allows different sub-populations to reach sexual maturity at different times
DNA (deoxyribonucleic acid)	the molecule that encodes genetic information. DNA is a double-stranded molecule held together by weak bonds between base pairs of nucleotides.
DNA sequence	the specific order of bases in a DNA molecule, whether in a fragment of DNA, a gene, a chromosome, or an entire genome
dormancy	a delay in the germination of viable seeds because of unfavourable environmental conditions
eclosion	the emergence of an insect larva from the egg or an adult from the pupal case
ecological amplitude	the range of environmental conditions in which an organism can survive and reproduce
ectoparasitoid	a parasitic insect with larval stages found on the external surface of its insect host
endoparasitoid	a parasitic insect in which larval development occurs within the body cavity of its insect host
entomo-fauna	insect species
epiphytic	one organism living within or upon another without causing harm
epistatic	a dependence relationship between genes; the product of one gene is unable to carry out its function because of the absence of another gene in the same organism
epitopes	separate antigenic areas within a given protein

erucic acid — 13 cis-docosadecenoic acid; a fatty acid having 22 carbons and one double bond and common to traditional rapeseed oil. Canola oil contains less than 2% erucic acid.

Escherichia coli (E. coli) — a bacterium found in the intestine of animals and humans used extensively in genetic engineering. Some strains can cause disease; the majority are harmless.

Exotic — non-native; refers to an organism that has been introduced into an area

expression (as in gene expression) — generation of a mRNA copy of a gene encoded in an organism's DNA

fibroblasts — irregularly shaped, branching cells distributed throughout vertebrate connective tissue

field trial — tests of the ability of new crop variety to perform under normal cultivation conditions

fitness — the genetic contribution of an individual to the next generation. The fundamental measure of evolutionary success.

flow cytometry — a technique for rapid automatic separation of suspensions of living cells into defined sub-populations

gamete — the products of meiosis; each gamete carries a single copy of the genetic information of the organism (i.e. a single set of alleles)

GE — genetically engineered (see GM)

gene — the fundamental physical and functional unit of heredity. A gene is a specific stretch of DNA located in a particular position on a particular chromosome that encodes a specific functional product (i.e. a protein or RNA molecule).

gene construct — a sequence of genes made by joining several genes together using recombinant DNA technology

gene flow — the movement of genes from one population to another

gene gun — a device for propelling DNA molecules into living cells

gene knockout strategy — an approach used to determine the function of a specific gene by inactivating (knocking out) that gene in the intact organism and studying the consequences of this modification

gene product — the biochemical material, either RNA or protein, resulting from expression of a gene

gene stacking	simultaneous presence of more than one transgene in an organism, usually a GM organism
genetic drift	the random change in the frequency of alleles in populations due to the small numbers of organisms involved
genome	the total DNA sequence of all the chromosomes in an organism, and thus the total genetic information of that organism
genomics	the study of genomes
genotype	the hereditary constitution of an organism
germplasm	a general term for the available pool of different genomes in a species
gill irrigation	the passing of water over gill filaments, the primary site of oxygen transfer from water to the blood in fish
glucosinolates	secondary metabolites found in plants of the mustard family (e.g. canola); their breakdown products can have goitrogenic properties in mammals
glycoalkaloids	toxic secondary metabolites found in the potato family
glycolysis	energy-yielding metabolic reactions by which sugars are converted to acids
GM	genetically modified; in this context, an organism into whose genome has been deliberately inserted one or more pieces of new DNA
GMOs	genetically modified organisms (see GM)
heat-labile	easily destroyed by heat
heterozygous	having two different alleles at a given locus of a chromosome pair
homology	structural similarity due to descent from a common ancestor or form
hybrid	offspring from a cross between genetically dissimilar individuals, often used to describe the progeny produced by matings between members of different species
immunoglobulin (Ig)	see antibody
immunoglobulin E (Ig E)	an antibody produced by an allergen which has specific structural and biological properties, in particular, ability to bind and activate mast cells and basophils, causing the release of chemical mediators resulting in clinical symptoms of allergy

in utero	within the uterus
in vitro	outside the living body; in a laboratory or test tube
in vivo	within the living body
insulin-like growth factor I	a peptide believed to be primarily secreted by the liver. It has growth-regulating, insulin-like and mitogenic activities. This growth factor has a major, but not absolute, dependence on somatotropin.
intellectual property (IP)	the legal rights associated inventions, artistic expressions and other products of the imagination (e.g. patent, copyright and trade-mark law)
introgression	movement of a new gene into a population
irradiation	a process involving use of low levels of radiation to reduce the presence of pathogens during the preparation of food products
leptokurtic	a statistical description of a population whose values are more heavily concentrated about the mean than in a normal distribution
lipogenesis	the conversion of carbohydrates and organic acids to fat
mass spectrometry	a sensitive physical technique for measuring the exact mass of a molecule and its fragments
mating system	the mode of transmission of genes from one generation to the next through sexual reproduction. Used in plants to refer to the amounts of self- and cross-fertilization.
meiosis	divisions of a nucleus preceding the formation of reproductive cells that contain one of each pair of chromosomes found in the parent cell
methanogenesis	the process of creating methane gas during metabolism
mitosis	the process of chromosome division and separation that takes place in a dividing cell, producing daughter cells of equivalent chromosomal composition to the parent cell
monophagous (oligophagous)	herbivores that feed on one or a small number of different closely related host plants
muscle ultrastructure	the structure of muscle tissue at the molecular level
mutagenesis	the process of changing the DNA base sequence at a specific site
mycorrhizae	a group of fungi that grow in close association with plant roots

nutrient	a substance required for health
ontogenetic delay	a delay in the course of growth and development to maturity
ontogeny	the course of growth and development of an individual to maturity
opercular region	the part of a fish in the head region, containing and protecting the gills, the tissue used in respiration in fish
operons	gene clusters under common control in bacteria
organoleptic	the taste and aroma properties of a food or chemical
outbreeding depression	a fitness reduction in hybrids produced by matings between individuals from two genetically distinct populations
outcrossing	mating between different individuals or genotypes
patent	a limited term monopoly, usually 20 years, granted to inventors of new, useful and non-obvious ideas with industrial application
phage	bacteriophage; a virus specifically attacking bacteria
phenotype	the sum total of observable structural and functional properties of an organism
plasmids	non-chromosomal pieces of DNA that code for a sub-set of cellular functions. Usually found in bacteria and fungi.
pleiotropic response	multiple changes to an organism's phenotype associated with a single change at the genetic level
pollination	the transfer of pollen between anthers (male sex organs) and stigmas (female sex organs) in seed plants
polyphagous	herbivores that feed on a wide variety of host plants from many different families
POnMTGH1 gene construct	a construct derived from sockeye salmon that consists of the metallothionein-B promoter fused to the full-length type-1 growth hormone gene
precautionary principle	a regulatory mechanism for managing environmental and health risk arising from incomplete scientific knowledge of a proposed activity's or technology's impact
prechondrocytes	precursors to cartilage cells
preweaning	prior to weaning, the time a young mammal stops nursing

prion — normal cell protein present on nerve cell membranes. It is found in most mammals, but its normal function is unclear. A mutated form of prion known as PrPsc is a disease-causing agent.

proteinase inhibitors — another class of proteins capable of inhibiting insect feeding

proteome — the complete complement of proteins made by a given species in all its tissues and stages

proximate analysis — chemical analysis of the main constituents of food

rate-limiting enzyme — an enzyme whose activity controls the overall flux through a linear sequence of reactions

recombinant DNA (rDNA) — DNA molecules created by splicing together two or more different pieces of DNA

reporter gene — a gene whose gene product is easily detected

restriction enzymes — DNA-cutting enzymes that recognize and bind to specific short sections of DNA sequence

rhizobacteria — bacteria found closely associated with plant roots

rhizosphere — the soil zone immediately surrounding a plant root system

salmonids — members of the fish family Salmonidae, including salmon, trouts and chars

secondary metabolite — a chemical produced by a plant that does not appear to have a direct role in its energy metabolism or growth; often restricted to particular species, tissues or developmental stages

secondary pests — those species within an ecosystem that are normally kept in check by natural enemies, but which, following certain agronomic practices (e.g. application of pesticides against a primary pest), reach densities that cause economic losses

seed bank — the population of dormant seeds below the soil surface

seed shattering — the spontaneous dispersal of mature seed from a plant following ripening

selectable marker gene — a gene whose product protects the cell containing it from a selection pressure such as a toxic chemical (e.g. antibiotic)

selfing — mating by a single hermaphrodite individual. Occurs commonly in plants.

single nucleotide polymorphism (SNP)	single-base variations in the genetic code between different individuals of the same species. SNPs occur at random throughout the genome. Researchers believe that knowing the locations of these closely spaced DNA landmarks will ease both the sequencing of the genome and the discovery of genes involved in major diseases.
smoltification	the combination of physiological, behavioural and morphological changes that salmonid fish experience when they migrate from fresh water rivers into the ocean
somaclonal variation	altered phenotype generated in plant tissues by extended growth *in vitro*; possibly a form of mutation
somatic cell nuclear transfer	the transfer of cell nuclei between cells in the body not involved in reproduction
stochastic processes	random processes
sympatry	organisms that occur in the same geographical region or area
syrphids	any fly of the family *Syrphidae* in the Diptera, typically having a colouration that mimics some bees and wasps
totipotency	the ability to regenerate a fully differentiated organism from a single somatic cell
transcription	the synthesis of RNA (ribonucleic acid) molecules concerned in translating the structure of DNA into the structure of protein molecules
transfection	the transfer into another cell of genetic material isolated from a cell or virus
transgene	a gene from one organism inserted into the genome of another
transposons	short stretches of DNA with the capacity to move between different points within a genome
triploidy	three copies of the genome in each cell rather than the normal two copies found in most plants and animals
vector	any organism or DNA construct that enables movement or transmission of another organism or gene
volunteer plant	crop plants that persist for a few seasons without deliberate cultivation

weed a plant that in any specified geographical region grows mainly in habitats markedly disturbed by human activities. Within the context of agriculture, weeds are generally unwanted plants that infest crops and reduce yields.

wide cross a sexual cross between distantly related species that normally would not breed

ACRONYMS AND ABBREVIATIONS

ALARA	As Low As Reasonably Achievable
ALARP	As Low As Reasonably Practicable
APHIS	Animal and Plant Health Inspection Service of the United States Department of Agriculture
BSE	bovine spongiform encephalopathy (mad cow disease) — a neurological disorder thought to be linked to the presence of mutant prions
BST	bovine somatotropin
Bt	*Bacillus thuringiensis* — a soil bacterium that produces a toxin that is deadly to some insects. Many strains exist, each with great specificity as to the type of insects it can affect.
CBAC	Canadian Biotechnology Advisory Committee
CCFL	Codex Committee on Food Labelling
CEPA	Canadian Environmental Protection Act
CFIA	Canadian Food Inspection Agency
DFO	Department of Fisheries and Oceans
DNA	deoxyribonucleic acid — the molecule that encodes genetic information. DNA is a double-stranded molecule held together by weak bonds between base pairs of nucleotides.
FAO	United Nations Food and Agriculture Organization
GC	gas chromatography
GE	genetically engineered (see GM)
GH	growth hormone
GM	genetically modified; in this context, an organism into whose genome has been deliberately inserted one or more pieces of new DNA
GMOs	genetically modified organisms (see GM)
HPLC	high performance liquid chromatography
ICES	International Council for the Exploration of the Sea
ICH	International Conference on Harmonization
IFT	Institute of Food Technologists
kb	Kilobases
LMOs	Living Modified Organisms
MTD	Maximum Tolerated Dose
NASCO	North Atlantic Salmon Conservation Organization
NBAC	National Bioethics Advisory Council (United States)
NBAC	National Biotechnology Advisory Committee (Canada)

NOAEL	No Observable Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
OMNR	Ontario Ministry of Natural Resources
PCPA	Pest Control Products Act
PCR	polymerase chain reaction
PMRA	Pest Management Regulatory Agency
PST	porcine somatotropin
rDNA	recombinant DNA
TSE	transmissible spongiform encephalopathy — despite distinctive individual features, a number of diseases of animals (scrapie, chronic wasting disease, transmissible mink encephalopathy), and humans (Creutzfeldt-Jakob disease, Gerstmann-Straussler-Scheinker syndrome, bovine spongiform encephalopathy, kuru), are considered to be transmissible spongiform encephalopathies
US NRC	US National Research Council
USDA	US Department of Agriculture
USFDA	US Food and Drug Administration
WHO	World Health Organization
WTO	World Trade Organization

MEMBERS OF THE EXPERT PANEL

Spencer C. H. Barrett, Ph.D., FRSC, Professor of Botany, University of Toronto

Dr. Barrett holds a doctorate in Botany from the University of California, Berkeley (1977) and was elected a Fellow of the Royal Society of Canada in 1998. His general research interests include plant evolutionary biology, evolutionary ecology and genetics, conservation biology, and plants and human affairs. His specific research has focused on such topics as plant reproduction, mating systems, biology of invading plants, and colonization genetics. He is the author of over 180 scientific publications and is co-editor of *Floral Biology: Studies on Floral Evolution in Animal-pollinated Plants* (1996).

Joyce L. Beare-Rogers, CM, Ph.D., FRSC, Ottawa, Ontario

Dr. Beare-Rogers received her doctorate in Lipid Biochemistry from Carleton University and joined the federal government's Food and Drug Directorate (now the Health Products and Food Branch) in 1956, where she worked until her retirement in 1992. Dr. Beare-Rogers is an internationally recognized authority in the areas of nutrition, lipids, fatty acids and dietary oils and was the first Canadian, and the first woman, to hold the office of President of the American Oil Chemists' Society. She was also President of the Canadian Society for Nutritional Sciences and is a Fellow of the Royal Society of Canada (elected 1989) and the American Institute of Nutrition.

Conrad G. Brunk, Ph.D., Academic Dean and Professor of Philosophy, Conrad Grebel College, University of Waterloo *(Panel Co-Chair*)

Dr. Brunk was awarded a doctorate in Philosophy from Northwestern University in 1974 and has held a faculty position at the University of Waterloo since 1976. His areas of specialization include applied and professional ethics, including environmental and bio-medical ethics, and conflict resolution. In addition to scholarly publications, including the book *Value Assumptions in Risk Assessment* (1991), he is well known for his reports on risk management frameworks for animal health and food trade. He served as Chair of the Royal Society of Canada's expert panel on the future of Health Canada's non-human primate colony in 1996.

Timothy Allen Caulfield, LL.M., Associate Professor, Faculty of Law and Faculty of Medicine and Dentistry, University of Alberta

Mr. Caulfield received his LL.M. degree from Dalhousie University (1993) and has been Research Director of the Health Law Institute at the University of Alberta since 1993. He is the co-editor of *Legal Rights and Human Genetic Material* (1996), *Canadian Health Law and Policy* (1999), and *The Commercialization of Genetic Research: Ethical, Legal and Policy Issues* (1999), and the author of numerous publications in scholarly journals, including "Regulating the Genetic Revolution" (1999).

Brian E. Ellis, Ph.D., Associate Director, Biotechnology Laboratory, Professor, Faculty of Agricultural Sciences and the Biotechnology Laboratory, University of British Columbia *(Panel Co-Chair)*

Dr. Ellis received his doctorate in Plant Biochemistry at the University of British Columbia in 1969 and was Head of UBC's Department of Plant Science from 1989 to 1999); his main interests are in the area of plant metabolism, especially lignin biosynthesis. His current projects include biochemistry of metabolic enzymes, signalling mechanisms whereby plants sense and respond to environmental changes, oxidative stress, and the genetic engineering of crop and forest plants. He teaches sustainable agriculture and professional communication as well as plant breeding and plant–microbe interactions.

Marc G. Fortin, Ph.D., Associate Professor and Chair, Department of Plant Science, McGill University

Dr. Fortin received his doctorate in Plant Molecular Biology from McGill University in 1987 and did post-doctoral work at the University of Chicago and the University of California at Davis. He has been at McGill as faculty member since 1990. His research focuses on applying molecular genetics approachs to better understand interactions between plants and microbes and was one of the initiators of the use of DNA markers for plant improvement. He has spearheaded the organization of two large inter-university research networks focusing on understanding plant productivity, and is an advisor to several provincial and national organizations dedicated to research in plant science.

Antony J. Ham Pong, M.B., F.R.C.P.(C) Paediatrics, Consultant in Allergy and Clinical Immunology, Ottawa, Ontario

Dr. Ham Pong, who has specialist training in Immunology and Allergy and in Paediatrics, has a clinical practice, is a lecturer in Paediatrics and an instructor for the Allergy/Immunology course at the University of Ottawa. He is a medical advisor to the Anaphylaxis Network of Canada, co-author of *Anaphylaxis: A Handbook for Schools* (1996), a frequent radio and TV commentator and guest lecturer on allergy issues. He has served on several task forces on Food Allergies and Anaphylaxis for Health Canada and other organizations. His professional publications include the recent co-authored study, *Common Allergenic Foods and their Labelling in Canada — A Review* (1999).

Jeffrey A. Hutchings, Ph.D., Associate Professor of Biology, Dalhousie University

Dr. Hutchings holds a doctorate in Evolutionary Ecology from Memorial University of Newfoundland (1991). Following research fellowships at Edinburgh University and the Department of Fisheries and Oceans (St. John's, Newfoundland), Dr. Hutchings has focused his work on the ecology, reproductive behaviour, genetics and population biology of marine and freshwater fishes. Among his 60 scientific publications, approximately one-half address environmental and genetic aspects of fish life histories, notably those of Atlantic salmon and other salmonids, and one third pertain to the collapse and recovery of Atlantic cod. An Associate Editor of *Canadian Journal of Fisheries and Aquatic Sciences* and *Transactions of the American Fisheries Society*, he has recently been appointed to the Committee on the Status of Endangered Wildlife in Canada (COSEWIC).

John J. Kennelly, PhD., Professor and Chair, Department of Agricultural, Food and Nutritional Science, University of Alberta

Dr. Kennelly holds a doctorate in Animal Nutrition from the University of Alberta (1980) and has been a Professor at the University of Alberta since 1987. He is a member of the Board of Directors of the National Institute of Nutrition and he has served as a member of the Alberta Science and Research Authority Biotech Task Force. In previous professional service, Dr. Kennelly was a member of the NSERC Animal Biology Grant Selection Committee for three years and Chair for one. He has also served as a member of the Editorial Board of *Animal Science* and was Chair of the American Dairy Science Association of Milk Synthesis Committee. Dr. Kennelly leads a research group at the University of Alberta that focuses on his primary scientific interest in nutrition and lactation physiology. Key areas of study are the nutritional and genetic factors that influence the biological efficiency of milk synthesis and its quality as a human food. Publications include over 120 refereed scientific papers, book chapters, conference proceedings as well as numerous extension articles.

Jeremy N. McNeil, Ph.D., FRSC, Professor of Biology, Université Laval

Dr. McNeil received his Ph.D. in Entomology and Ecology at North Carolina State University in 1972 and since then has been a professor in the Biology Department at Université Laval. His research is in chemical and behavioural ecology, looking for ecologically and socially acceptable alternatives to conventional pesticides. He is the author of over 130 scientific publications and serves on a variety of national and international scientific committees. He is also active in the public awareness of science, speaking to more than 2000 children annually. He was elected to the Royal Society of Canada in 1999.

Leonard Ritter, Ph.D., Executive Director, Canadian Network of Toxicology Centres and Professor and Associate Chair, Department of Environmental Biology, University of Guelph

Dr. Ritter holds a doctorate in Biochemistry from Queen's University (1977) and has been a professor at the University of Guelph since 1993. He is the founding Executive Director of the Canadian Network of Toxicology Centres, based at the university, which involves the coordination of a national, multi-disciplinary toxicology research program. From 1977 to 1993, he worked in various positions at the Health Protection Branch of Health Canada, with responsibilities for the regulation of pesticides and veterinary drugs. He has publications, technical reports or responsibilities on international bodies in the areas of pesticides residues in foods, pesticides exposure and cancer, persistent organic pollutants, food additives, endocrine modulating substances, and the use of hormones in food production.

Karin M. Wittenberg, Ph.D., Professor and Head, Department of Animal Science, University of Manitoba

Dr. Wittenberg has a doctorate in Ruminant Nutrition from the University of Manitoba (1985), where she is now a professor and currently serves as Head of the Department of Animal Science and the Director of the Ruminant Research Unit. She was an invited member (1995–99) of the Committee on Animal Nutrition of the US National Research Council, including its Biotechnology Advisory Council on Microbial Products as Livestock Feed, and for 10 years a member of the Expert Committee on Animal Nutrition of the Canadian Agricultural Services Coordinating Committee. Her research is in the areas of forage utilization, harvest and post-harvest practices, microbial processes in forage, and the use of forage additives; among her publications are a co-authored book, *The Role of Chromium in Animal Nutrition*, and a review article on "the role of additives in hay production."

R. Campbell Wyndham, Ph.D., Professor and Chair, Department of Biology, Carleton University

Dr. Wyndham received his doctorate in Biology from the University of Calgary in 1982 and has been a member of both the Institute of Biochemistry and the Institute of Biology at Carleton since 1987. He specializes in studies of microbial ecology, including the ecology and genetics of pollutant-degrading bacteria (particularly in wastewater), and also is increasingly active in applying molecular techniques to understanding how genetically modified microorganisms behave in agricultural ecosystems. In the course of studying the ecological risks of biotechnology, his laboratory is developing rapid and simple soil microcosm and DNA-detection protocols to assess gene transfer frequencies. For the past 10 years, he has contributed expert advice to federal departments on the new substances notification regulations for products of biotechnology under the Canadian Environmental Protection Act.

Rickey Yoshio Yada, Ph.D., Professor and Assistant Vice President Research, Agri-Food Programs, University of Guelph

Dr. Yada was awarded a doctorate from the Department of Food Science at the University of British Columbia in 1984. He has been a faculty member at Guelph since that time, has served as Chair of the Department of Food Science, and currently is the Assistant Vice President Research, Agri-Food Programs. His primary research focus is on structure–function relations of food-related proteins, and he has specialized in the study of potatoes. He has been a member or chair of numerous NSERC research awards panels and committees and is currently one of the Life Science Group Chairs for NSERC, and a member of the Committee on Research Grants. He was Editor-in-Chief of *Food Research International Journal* from 1992 to 1998 and now is the North American Editor for *Trends in Food Science and Technology*. He is the author of over 100 refereed journal publications and the co-editor of two major books in his field, *Functional Properties of Food Components* (1998) and *Protein Structure–Function Relationships in Food* (1994).