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HEALTH CANADA
HEALTH PRODUCTS AND FOOD BRANCH
FOOD DIRECTORATE

- FINAL REPORT -

TECHNICAL DISCUSSION ON THE HEALTH AND SAFETY ASPECTS OF THE GOVERNMENT OF CANADA ACTION PLAN

TUESDAY, APRIL 30, 2002
CHATEAU CARTIER
GATINEAU, QUEBEC

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1. INTRODUCTION AND BACKGROUND

The Government of Canada must ensure that it will have the necessary scientific and regulatory capacity to adequately regulate future products derived from biotechnology. In November 2001, the Government of Canada published an action plan in response to the Expert Scientific Panel of the Royal Society of Canada report entitled: Elements of Precaution: Recommendations for the Regulation of Food Biotechnology in Canada[1]. The action plan describes specific actions and projects that the federal government intends to carry out in response to the Expert Panel's recommendations. In this action plan, Health Canada and other departments also commit to collaborate with external experts to further enhance the regulatory processes and protocols.

Consistent with these commitments, Health Canada held a technical discussion on April 30, 2002.

The main objectives of the session were:

- to seek input on how to further develop Health Canada's ongoing or planned research projects identified in the action plan dealing with the health and safety aspects of genetically modified foods (GM-foods), and
- to identify new activities to be initiated, ensuring the continued technical contribution and collaboration of external experts with the Government of Canada.

The session format included presentations, breakout sessions and plenary reports. Presentations and discussions were grouped around five themes: molecular characterization; allergenicity; toxicology; nutrition and long-term surveillance. During the breakout sessions, participants were asked to identify the research needs in the area being discussed and prioritize these needs. They were then asked to identify the research that is currently being carried out by other organizations and identify possible collaborative work opportunities. For those research needs not currently being undertaken, participants were asked to identify how these needs could be met.

Approximately 50 experts attended the technical discussion from academia, the former Royal Society Expert Panel on the future of food biotechnology, the Canadian Biotechnology Advisory Committee (CBAC), non-governmental organizations and industry.

This report provides an overview of the meeting including presentations highlights, key findings and next steps.

[1] http://www.hc-sc.gc.ca/english/protection/royalsociety/index.htm

2. WELCOME AND OPENING REMARKS

Karen Dodds, PhD, Director General, Food Directorate, Health Canada

Dr. Dodds welcomed the participants and outlined their role in identifying and prioritizing research needs in the various areas as well as identifying possible partnership opportunities.

She noted that the workshop session would focus on the scientific elements of the action plan and, more specifically on those elements related to human health and safety. She also indicated her wish for this session to be a day of exploration and information sharing, having as the primary objectives to encourage the understanding of the science as well as collaboration building among experts in different yet complementary fields.

3. PRESENTATIONS

3.1 Overview of the Codex Guidelines for the Conduct of Food Safety Assessment of Foods Derived from rDNA Plants

Paul Mayers, Acting Associate Director General, Food Directorate, Health Canada

Mr. Mayers provided an update on the work of the Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology and an overview of the Guidelines for the Conduct of Food Safety Assessment of Foods Derived from recombinant DNA (rDNA) Plants recently adopted by the Task Force. These guidelines are available on the Codex Alimentarius website at www.codexalimentarius.net.

3.2 Molecular Characterization: Key Consideration in the Safety Assessment of Novel Foods

William Yan, PhD, Chief, Evaluation Division, Bureau of Microbial Hazards, Food Directorate, Health Canada

Dr. Yan described the data requirements with regards to the molecular characterization component of the safety assessment of foods derived from biotechnology. The potential benefits of profiling techniques (genomics, proteomics, etc.) were discussed, noting however, that these new methodologies needed first to be further developed and validated before they can be applied to the assessment of novel foods. Further information is available on the Food Directorate website at www.hc-sc.gc.ca/food-aliment/ under the heading "Novel Food".

Dr. Yan also discussed the use of marker genes, including the concerns of the public regarding the use of antibiotic-resistance genes. He noted that alternative transformation technologies that do not result in the presence of antibiotic resistance genes in foods are recommended for the development of future products.

3.3 DNA microarray technology: applications and uses

Franco Pagotto, PhD, Research Scientist, Bureau of Microbial Hazards, Food Directorate, Health Canada

Dr. Pagotto provided an overview of the research that is currently underway within Health Canada in collaboration with other federal regulatory agencies to develop detection and identification procedures for genetically modified organisms (GMO) based on DNA chip technology. The researchers are also evaluating and optimizing such systems for the analysis of GMO in processed foods, food ingredients and related samples. Please refer to Appendix I for further details.

3.4 Toxicology and Allergenicity - Overview of Current and Future Research

Rekha Mehta, PhD, Acting Chief, Toxicology Research Division, Bureau of Chemical Safety, Food Directorate, Health Canada

Dr. Mehta brought to the forefront the challenges and current research in the field of allergenicity, including the conclusions from the Health Canada Workshop on Animal Models to Detect Allergenicity to Foods and GM-Products. She also provided an overview of the issues and ongoing research related to toxicological testing, including the issue of whole food testing. Please refer to Appendix II and III for further details.

3.5 Dietary Phytoestrogens: Safety, Nutritional Quality and Health Considerations

Ghulam Sarwar Gilani, PhD, Senior Research Scientist, Bureau of Nutritional Sciences, Food Directorate, Health Canada

Dr. Gilani reviewed the ongoing research into the possible health benefits and potential adverse effects of dietary phytoestrogens. Data from two rat studies (a 16-week study with Sprague-Dawley rats and a life span study with Stroke-Prone Spontaneously Hypertensive rats) conducted in Health Canada laboratories to assess safety of soy isoflavones were presented. He also provided participants with an overview of the planned research related to the safety assessment of dietary phytoestrogens. Please refer to Appendix IV for further details.

3.6 Update on the Biotechnology Surveillance Project

Rita Beregszaszy, Project Manager, Centre for Surveillance Coordination, Health Canada

Ms. Beregszaszy provided a brief update on Health Canada's Biotechnology Surveillance Project, specifically detailing the activities regarding post-market surveillance of GM-foods. Additional information on this project is available on Health Canada's website at http://www.hc-sc.gc.ca/pphb-dgspsp/csc-ccs/biotech_e.html.

She also mentioned the International Conference on Post-Market Surveillance of Genetically Modified Foods that will be sponsored by Health Canada's Centre for Surveillance Coordination. The purpose of this conference is to discuss the technical issues, challenges and opportunities in the area of post-market surveillance of genetically modified foods. This workshop will be held in Ottawa in October 2002. Information on the conference is available at http://www.gmfoodsurveillance.org.

4. BREAKOUT GROUP DISCUSSIONS

4.1 Molecular Characterization

The top research need identified by the participants in this field was the development of gene expression baseline data for plants. The establishment of the range of expression inherent to any given crop will enable a more precise determination of the extent of the changes following the expression of the transgene(s) in genetically modified lines. It was suggested that obtaining this type of data for the major agricultural crops would be a good starting point. In order to obtain this data, participants generally agreed that tools (eg. bioinformatics), methodologies and networks as well as international standards for their application, need to be developed further.

Generally, participants agreed that the approach should be to target specific hazards and work back from there. They also noted that research is required to expand the use and development of molecular tools for post-market surveillance.

4.2 Allergenicity Considerations

Participants agreed that there are many areas related to the determination of possible allergenicity that required further research. One such area was the development of real-time rapid assay techniques for the detection and prediction of all allergens (other than the top 8) in whole foods or food ingredients. The group also acknowledged that a better understanding of the biochemical characteristics of proteins would help in determining their capability to elicit an allergic response. This characterization is required for both allergenic and non-allergenic proteins in order to further our understanding of allergenicity triggers and enable us to distinguish between them.

Furthermore, the participants identified the need for the development of targeted and specific serum screening methods, including the development of serum banks. They also noted that a better understanding of the dose threshold for an allergic reaction as well as the threshold for sensitization is required.

The participants also emphasized that the development of validated techniques and animal models would be beneficial. However, they concluded by stating that although further research is required in many areas a lot of work is currently being undertaken internationally to address many of these issues.

4.3 Toxicological Considerations

Participants addressed the questions of acute versus chronic toxicological effects and generally agreed that both of these must be considered in the assessment of the safety of novel foods. However, research should focus on the chronic effects in order to address the knowledge gaps in this area. Thus far, the focus has been on the acute effects since the changes in the food products that have been introduced to date have been relatively simple, however some future products are

expected to be more complex.

To address the toxicological requirements for these future products, participants suggested the use of a knowledge-based tiered approach. This would require the establishment of baseline data for non-GM-products in order to identify any altered levels in a GM-product which are outside the range of natural variation, whether this change is intended or unintended. If the level of exposure is outside the normal range, depending on the compound in question and its potential exposure to humans, the level of toxicological testing that would be required could be determined. This approach would also require the development of standardized methods.

Participants also identified the need to develop more sensitive and validated biomarkers for the evaluation of possible reproductive effects, immunotoxicity, carcinogenicity and neurotoxicity. It was also acknowledged that there are studies and information that exist, such as animal models that are currently used for the assessment of nutritional considerations, that may be applied to the toxicological safety assessment. These methods may also lead to the creation of standardized testing and could aid in the development of protocols for whole food testing. It was also suggested that toxicologists and regulators from the pharmaceutical and agricultural-chemical industry might have information about biomarkers that may be applied to foods. The group also proposed that data from certain known toxic chemicals could be used as endpoints for testing novel foods.

4.4 Nutritional Considerations

The group discussed the need for standardized methods for nutrition testing in animals as well as standardized bioactive components for anti-nutrient testing for those crop species (e.g. broccoli and papaya) where animal models do not exist. One of the issues raised was the need to define which components must be evaluated when reference is made to a comprehensive assessment of nutrient composition and bioactive components.

Participants identified the need for the creation of a reference database for baseline nutrient composition that would indicate the level of natural variation. The need for dietary intake data that reflects the food habits of Canadians was also expressed. The purpose of this data would be to determine the usual levels and patterns of consumption of particular foods and food groups by Canadians, and possibly the exposure to genetically modified foods or potential contaminants / toxins in foods.

Generally, the group agreed that the nutritional assessment must take into account the nature of the modification of the product as well as the potential risks associated with these changes. They stated that the ability to assess the impact of the nutritional change of new products is complicated by the need for comprehensive testing, including genetic, animal, human and population considerations.

4.5 Post-Market Surveillance

The group identified the need to assess the cumulative effect of exposure to GM-foods, however a mechanism must be developed to identify adverse reactions to relate these effects to GM-foods. Research is also required on the food consumption pattern and dietary intake of GM-foods. Furthermore, baseline data showing what Canadians are eating must be established in order to test the hypotheses. Some participants questioned the use of the term "baseline" since the population has been exposed to GM-foods for some time.

To address these issues, the participants identified the need to examine food consumption patterns to determine the dietary intake of GM-foods and to relate the outcomes to the exposure. It was also noted that in order to implement the long-term surveillance of GM-foods the current research gaps must be addressed (i.e., gathering baseline data and other pre-market assessment research needs identified in other breakout group discussions, etc.) as well as the establishment of reporting mechanisms and standardized evaluation protocols. However, it was observed that many opportunities for collaboration with academia exist. Also, there are surveillance approaches developed for other commodities whose methodologies could be extended to GM-foods.

The International Conference on Post-Market Surveillance of Genetically Modified Foods, sponsored by Health Canada, will provide an opportunity to explore and discuss the technical issues, challenges and opportunities surrounding this issue (Ottawa, October 16 -17, 2002).

5. NEXT STEPS AND CONCLUDING REMARKS

In each of the topics discussed, next steps were identified to further the research in the area of GM-foods (refer to the summary table below). One of the common needs identified to many of the areas, was the development of baseline data. This was recognized as an opportunity for collaboration with academia as there are many research projects that are either planned or underway. The need for international standards for methods and protocols in many fields was also observed. This work must be done in collaboration at an international level, therefore the government will ask the OECD Task Force on Novel Foods and Feeds to explore the need for whole food testing protocols for toxicological and nutritional endpoints.

The molecular characterization of genetically modified foods is a field in which additional tools are still being developed. The need for baseline data was identified as a major gap and the establishment of baseline data for major agricultural crops such as corn was suggested as a first step.

In the field of allergenicity, most of the work is currently being undertaken at an international level. A gap analysis is however required before assessing where Canadian research efforts should be focussed. Regarding to the development of serum banks, International Life Science Institute (ILSI) is examining this issue and opportunities for collaboration with them will be explored.

The group discussing toxicological considerations proposed that the existing knowledge (e.g., animal models for nutritional assessment) should be reviewed by an expert committee in order to develop standardized testing methods. This could then potentially lead to the development of whole food testing protocols. The Canadian Agri-Food Research Council (CARC) and/or the Society of Toxicology Canada were suggested as possible fora for such a committee which would include academia, industry, government and other experts.

With regard to research needs in the field of nutrition, Health Canada was identified as the possible lead for a survey on dietary intake and consumption data. Health Canada was also encouraged to collaborate with academia, industry and related groups such as National Institute of Nutrition (NIN) to meet the needs identified during the discussions.

The group discussing the topic of post-market surveillance concluded that even though Health Canada and Environment Canada both have projects underway, a larger scale study on potential health effects of GM-foods should be undertaken in collaboration with the scientific community. It was also noted that there are many existing studies that may contain relevant data or methodologies that could be applied to the surveillance of GM-foods.

Paul Mayers addressed the group and thanked them for their participation. He noted that as a first step, Health Canada would review the outcomes of the discussions and explore through CARC, the opportunities for developing a strategy to encourage further collaboration between governmental and non-governmental experts to move forward on the issues identified.

Table 1. Summary of the Needs Identified in the Different Breakout Groups

Molecular Characterization	*Allergenicity*	*Toxicology*	*Nutrition*	*Post-Market Surveillance*
Development of gene expression baseline data for major crops	Development of real-time rapid assays for assessment and detection of allergens	Development of baseline data for non-GM-products (i.e. data on the range of natural variation)	Creation of a reference database for baseline nutrient composition	Collection of data on food consumption patterns and dietary intake habits of Canadians to establish exposure to GM-foods
Further development of tools (eg. bioinformatics), methodologies and networks to obtain baseline data	Development of serum banks to be used to evaluate potential allergenicity of proteins	Development of standardized toxicological tests	Development of standardized methods for nutritional testing in animals	Establishment of reporting mechanisms for adverse effects
Development of international standard techniques for the application of these tools and methodologies	Development of targeted and specific serum screening methods to assess potential allergenicity of proteins	Development and validation of sensitive biomarkers for the evaluation of possible reproductive effects, immunotoxicity, carcinogenicity and neurotoxicity of new substances	Defining the nutrients and bioactive components to be examined for various crop species	Development of standardized protocols for assessment of reported adverse effects
Expanding the use and development of molecular testing methods for post-market surveillance	Characterization of sensitization threshold and allergic reaction threshold	Evaluation of methods used in other fields, such as animal models used in nutrition research, for its potential application in toxicology studies	Collection of data on food consumption patterns and dietary intake habits of Canadians to establish exposure to GM-foods	
	Development of animal models to assess potential allergenicity of proteins	Collection of information on biomarkers used in the pharmaceutical and ag-chemical sectors for potential application to foods		

6. EVALUATION

WHAT WENT WELL

- Facilitators were good (5)
- The presentations before the breakout sessions were focused, covered the appropriate information and helped the participants become familiarized with the different areas of research (4)
- Wide-ranging and informed discussions (4)
- Nice location/facilities (3)
- Organized (3)
- The breakout groups were well organized and a great deal of good constructive discussions went on (2)
- Good representation and participation from industry, university and government (2)
- Pointed out research gaps effectively (2)
- Good selection of experts (2)
- Format was conductive to exchange of ideas in an organized fashion
- Everybody had a chance to speak his or her mind
- Everyone was committed to the improvement of the science
- Everyone was polite and considerate
- Good overview of initiatives
- Brief introductory talks
- Free parking

WHAT COULD BE BETTER

- Materials and supportive websites should have been provided to participants prior to the meeting (4)
- The time frame was too short; there was not enough time to tackle the complex problems (4)
- Will there be actions taken after this exchange of ideas? (2)
- Need to assign responsibility (2)
- Greater representation from academia to understand the current state of the research (2)
- The discussion groups on toxicology/allergenicity in the afternoon needed to be smaller
- The government should provide leadership in resourcing the research needed to close the gaps
- The sessions should have focused on pre-written issues
- Intent of post-market surveillance or objectives
- Ability to define negative outcomes against a background of ever changing dietary habits without full appreciation of background intakes
- Implementation of standardized whole food testing
- Establish matrix on follow-up items
- Not a lot of true experts in a specific area except those from Health Canada
- It seems that this has been done before and few really concrete conclusions arise
- This should have been done earlier (i.e. before the introduction of GM-foods in our food supply)
- What about the environmental assessment portion of the issue of GM-foods?
- Don’t loose the momentum created at this meeting
- Provide report as soon as possible

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APPENDIX I. DNA MICROARRAY TECHNOLOGY: APPLICATIONS AND USES FOR THE HEALTH AND FOOD SAFETY OF GM-FOODS

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Summary:

Foods produced by genetic engineering technology are now appearing on the market and many more are likely to emerge in the future. The detection of GMOs is growing in importance in response to resistance by consumers to products containing ingredients manipulated by recombinant DNA techniques due to concerns with (i) possible health-related issues resulting from the altered biochemical composition of GM-foods, or the inadvertent introduction of allergens; (ii) the control of proprietary GM-products, and (iii) environmental protection issues. Recently, the European Union and many other industrialized nations have announced the implementation of new regulations requiring that foods containing more than 1% GMOs must be clearly identified on the label and there is increasing pressure in North America for governments to adopt regulations on labelling. Such a policy will have dramatic social and economic consequences for the Canadian agri-food sector, which is a major exporter of agricultural commodities implicated in the GMO issue. In Canada, some of the major crops that have been the focus of genetic modifications are corn (Bt, Liberty Link and Roundup Ready), potato (pathogen resistance) , soybean (Roundup Ready) and canola (Liberty Link, Roundup Ready, Bromoxynil tolerance, Male sterility, Restorer, High Laurate). Present methods used for detection of genetic modifications in foods and related samples are time-consuming and labourious. In order to judiciously regulate the labelling of GMO status, novel techniques are required to detect the possible presence of many different genetic events quantitatively.

The lack of validated analytical methods in Canada makes it difficult at present to develop a regulatory testing program for monitoring GMOs in foods. Currently, there are no analytical technologies able to simultaneously detect the large number of targets encountered in GMOs, greatly limiting our current analytical capability. Therefore, the development of the DNA chip technology will provide a valuable tool for the implementation of a comprehensive regulatory program. The aims of the project include (a) to develop comprehensive detection and identification procedures for genetically modified organisms (GMO) based on DNA chip technology and (b) to evaluate and optimize DNA chip-based detection systems for the analysis of GMO in processed foods, food ingredients and related samples, including GM soy, corn, potatoes and canola.

A leading edge technology will be developed to enable such agencies as CFIA and the Agri-food sector to regularly monitor foods and related commodities for the presence of GMOs to meet national and international labelling requirements. There will be improved access of Canada's Agri-food sector to foreign export markets by virtue of the ability to determine the complete GMO status of commodities utilizing this new analytical capability. While there is no proven negative impact on human health associated with approved GMOs in foods, many concerns

persist creating an increasingly negative climate for advancement in this field. The availability of analytical technologies allowing responsible agencies to routinely monitor the GMO status of foods will demonstrate to the public that science and technology is being diligently managed in this country as well as preventing the inadvertent addition of health-threatening components such as allergens to foods.

Microarray (DNA chip) technology has evolved into a feasible technology for application to DNA analysis and features such as small scale, accuracy and speed make it an ideal technology as alternative to PCR for the detection and identification of GMO's in food. Oligonucleotide arrays formed on chip surfaces composed of 12-15 bp DNA fragments can be hybridized with complementary target DNA present in a test sample and visualized quantitatively using a fluorescent dye in a commercially available image analysis system. The proposed project will involve the identification of oligonucleotide sequences for all major events encountered in corn, soy, canola and potatoes, and probes will be synthesized for immobilization in arrays on chip surfaces. Conditions for probe immobilization and subsequent hybridization with target DNA will be optimized. Target DNA (DNA extracts from test samples) will be fragmented by mechanical (ultrasonication), enzymatic (DNAse digestion) or chemical (metal-ligand hydrolysis) means to render fragments of ideal size for efficient interaction with the immobilized probes. Procedures for recovery of DNA from diverse samples, such as seeds, whole crops and finished foods (e.g., corn chips and honey) will be developed. This will include examination of automated DNA extraction technology for simultaneously processing large numbers of samples. The assay format will be refined for the quantitative detection of GMOs in samples, in order to determine the level of GMO in the bulk sample. This will be achieved through consistency in high quality preparation of the DNA arrays as well as exploitation of the array scanner software in performing data analysis. Finally, the optimized assay format will be evaluated in the analysis of a variety of foods, crops and related samples to determine the performance characteristics of the technique (sensitivity, specificity and reproducibility).

The team working on this project include Dr. Jeffrey Farber (Health Canada), Nathalie Corneau (Health Canada), and Dr. Burton W. Blais and Amalia Martinez (Canadian Food Inspection Agency).

APPENDIX II. TOXICOLOGY AND ALLERGENICITY - ISSUES, CHALLENGES, AND CURRENT RESEARCH IN THE FOOD DIRECTORATE

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Issues

Under the Food and Drugs Act, the Food Directorate of Health Canada has the responsibility for safety evaluation of new food products such as those developed through genetic manipulation (genetically modified foods or GMF) or food fortification. In Canada, GMF products undergo comprehensive review by federal departments, and are regulated as "novel foods". The process for safety assessment of novel foods is based upon principles developed through technical stakeholder and expert consultations carried out by the World Health Organization (WHO), the Food and Agriculture Organization (FAO) of the United Nations, and the Organization for Economic Co-operation and Development (OECD). Current GMF on the market consist mostly of plants with simple genetic modifications such as addition of one or two traits. However, the next generation of GMF are likely to emerge from both plant and transgenic animal origin, and through modifications of more complex traits and altered chemical and nutritional composition. The recognition of a potential for an increased complexity of future GMF as well as close public and expert scrutiny, has resulted in re-examination of current regulatory guidelines for products of food biotechnology in Canada and worldwide. Thus, Health Canada, Agriculture and Agri-Food Canada, and Environment Canada convened a Royal Society of Canada (RSC) Expert Scientific Panel in December 1999 to provide advice on scientific issues related to safety and human health effects of new GMF. The RSC Expert Panel (2001) made a number of recommendations including:

- refining the assessment of potential allergenicity of GMF ;
- improving the criteria for toxicological testing for GMF ;
- developing and validating of whole food testing protocols for GMF with multiple compostional/nutritional modifications.

Similar recommendations have come through several important reviews of assessment strategies for products of biotechnology conducted by expert groups internationally, for example, the American Medical Association (AMA), the British Royal Society and the US National Academy of Sciences. Health Canada has developed a detailed action plan to address the recommendations of the RSC Expert panel, including an R&D Program which supports research activities to continue to ensure the safety of new food products being developed through biotechnology into the 21st century.

Challenges and Current Research

1. Allergenicity

Until recently, the human health issues for GMF have related specifically to potential allergenicity of the presence of minute amounts of one or several novel proteins expressed by recombinant DNA in GM-food crops, and novel proteins as enzymes used for food processing. The present approach for assessment of potential allergenicity relies on data relating to expression level of the novel protein in the GMF, its stability to processing and digestion in the mammalian gastrointestinal tract, homology of the amino acid sequence with that of known food allergens, and immunochemical binding of the new protein with IgE from the serum of individuals known to be allergic to the source of the transferred gene.

With reference to enhancing allergenicity assessment, activities at Health Canada include participation in FAO/WHO international expert consultations (2000, 2001), and as a lead in the development of an annex on allergenicity for Codex Guidelines on the Safety Assessment of Recombinant-DNA Plants. The FAO/WHO Expert Consultation (2001) recommended a hierarchical approach to allergenicity assessment for GMF, which suggested that animal models, when sufficiently developed and validated, could contribute valuable information to the process of identifying potential sensitization to food proteins. In response to this recommendation, Health Canada scientists organized a 2-day workshop in November 2001 in Ottawa. Several prominent scientists participated in this Workshop to review the current status and further development of animal models of allergenicity, and to discuss the potential applicability of these models in predicting the allergenicity of foods including GMF. The Workshop Proceedings have been submitted for publication in Environmental Health Perspectives (2002, In Press). An Executive Summary of these Proceedings is attached in Appendix III. The Workshop participants concluded that although there is no single animal model that ideally meets the requirements, each of these models has merits which when further validated may contribute to the overall assessment of allergenicity for GM-derived protein products. Alternate methods such as the development of *in vitro* assays to predict functional T-cell epitopes in individuals previously unexposed to proteins of concern were also discussed. The most useful application of this technique may be the development of hypo-allergenic substance variants which would then help reduce the overall allergenic potential of GM-proteins and other novel substances.

2. Toxicology and Whole Food Testing

Current Health Canada regulatory protocols require complete toxicological testing, including pharmaco-kinetic studies, and genotoxicity, carcinogenicity, reproductive and teratology tests if a novel protein or other component in a GMF is present at a level outside a currently established range. Substantial equivalence is a regulatory tool, underpinned by sound science and strongly supported by OECD. It is used as a starting point, and not as a decision threshold, for the assessment process. There are many elements to the evaluation approach, including: (a) phenotypic assessment; (b) compositional analysis for naturally occurring toxins and allergens, and (c) equivalence of protein/gene introduced. In the most recent Joint FAO/WHO expert consultation on Foods derived from Biotechnology (2000), it was concluded that while the "substantial equivalence" concept is a robust framework for the safety assessment, some aspects of the steps in the safety assessment process could be refined to keep abreast of developments in

genetic modification technology. For GM crops with more complex traits where, for example, the purpose is to improve micro-nutrient levels, an extensive toxicological and nutritional assessment of the GM-foods may be required. Another issue regarding the "substantial equivalence" framework is that the comparison of the levels of key nutrients and toxicants in a GM variety with that of a traditional counterpart is limited to the presence of known components. This precludes the identification of unknown components of toxicological significance which might emerge in the modified food.

A key challenge for toxicological testing or the conduct of long-term feeding studies in animals for GM-foods compared to the safety assessment of pharmaceuticals or food additives for example, is that a whole food matrix is a complex mixture of compounds that can be tested only at dose levels that are tolerable for palatability and maintenance of normal growth of the test animal. Such doses may not reach levels that are as high as either the anticipated human exposures or to detect any subtle adverse effects, thus making the data obtained from conventional toxicological tests unreliable for human health risk assessment. Validated study protocols that are biologically and statistically acceptable for the safety assessment of any food as a whole food matrix are currently lacking, and hence, practical difficulties exist in application of conventional toxicological tests as routine safety assessment tools for GM-foods. Therefore, Health Canada as well as international organisations (OECD, FAO/WHO) have recognised a need to support research for the design and development of practical and scientifically sound *in vivo* models for toxicity testing of whole foods.

Health Canada scientists, in collaboration with investigators at the Canadian Food Inspection Agency, Department of Fisheries and Oceans, and Universities of McGill, Manitoba and British Columbia, are addressing this challenge by conducting studies in Sprague Dawley (SD) rats using soy isoflavones as a model class of compounds. Levels of isoflavones may be affected either primarily or secondarily by genetic modifications made to soy products. The effects on metabolism, reproduction, general and neural development, immune system and cancer induction are being assessed. Any potential adverse effects of ingested GM-fish is similarly being determined using a SD rat model. *In vitro* assays, using soy isoflavones and genomic methodologies, are also being developed with gene expression and mutagenic activation / genotoxicity as endpoints. All these studies include analysis of events related to aberrant gene expression using the leading edge molecular diagnostic technologies such as DNA and protein micro-arrays. The rationale for such analysis is to provide opportunities for identification of molecular biomarkers in target cells and tissues of the *in vivo* and *in vitro* model systems that are sensitive and specifically related to clinical or disease outcomes. Development of such biomarker based toxicity testing methods, by virtue of their sensitivity and specificity to detect subtle grades of toxicity, may then provide test models for assessing the safety and nutritional quality of a GMF, either on the whole food, or food constituent or component in question.

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APPENDIX III. HEALTH CANADA WORKSHOP ON ANIMAL MODELS TO DETECT ALLERGENICITY TO FOODS AND GM-PRODUCTS (NOVEMBER 2001, OTTAWA, ONTARIO, CANADA)

Helen Tryphonas[1], George Arvanitakis[2], Elizabeth Vavasour[1] and Genevieve Bondy[1].

Health Products and Food Branch[1], and Healthy Environments and Consumer Safety Branch[2], Health Canada, Ottawa, Ontario Canada K1A 0L2.

EXECUTIVE SUMMARY

Respiratory allergy and allergy to foods continue to be important health issues. There is evidence to indicate that the incidence of food allergy around the world is on the rise. Current estimates indicate that approximately 5% of young children and 1-2% of adults suffer from true food allergy (see Kagan in this Monograph). While a large number of *in vivo* and *in vitro* tests exist for the clinical diagnosis of allergy in humans, there is a lack of validated animal models of allergenicity. This deficiency creates serious problems for regulatory agencies and industry that need to define the potential allergenicity of foods prior to marketing. The emergence of several biotechnologically derived foods and industrial proteins, and their potential to sensitize genetically predisposed populations to develop allergy, has prompted health officials and regulatory agencies around the world to seek approaches and methodologies to screen novel proteins for allergenicity.

One such approach was proposed initially by the International Life Science Institute (ILSI) Allergy and Immunology Institute in collaboration with the International Food Biotechnology Council (IFBC) (Metcalfe et al, 1996) and was subsequently modified by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) (FAO/WHO, 2001). The FAO/WHO 2001 Decision Tree Approach for the Evaluation of Allergenicity of Genetically Modified Foods recommended a hierarchical approach to safety assessment (reviewed by Metcalfe et al, 1996, Taylor and Hefle, 2001 and FAO/WHO, 2001). One of the recommendations in this approach was that animal models, when sufficiently developed and validated, could contribute valuable information to the process of identifying potential sensitization to food protein. Based on this recommendation, Health Canada undertook to organize a 2-day Workshop held in Ottawa in November 13-14, 2001. Several prominent research scientists, currently developing animal models of allergenicity convened to a) review the current status of such models, b) identify areas which need further development and c) discuss the role these models may play in predicting the allergenic potential of foods including the genetically modified food products. Data were presented on several murine and non-murine animal models currently under development. In addition emerging approaches including the identification of antigenic epitopes on human allergens using the HLA transgenic mouce and a human dendritic cell-based method to identify CD4+ T cell epitopes in potential protein allergens were presented. This monograph consists of selected papers which are relevant to the

development of animal models. A summary of key issues discussed at this workshop follows.

Animal models presently under development include the Brown Norway rat, the BALB/c mouse and a transgenic mouse strain engineered to produce class II human leukocyte antigen (HLA) molecules. The potential to sensitize BALB/c mice systemically via intraperitoneal injections of proteins has been explored in a series of experiments detailed by Kimber and Dearman (see paper in this monograph). This approach favors the initiation of vigorous humoral immune responses of the IgG and IgE class and could be a useful model to screen novel proteins for their allergenic potential. The Brown Norway (BN) rat is a high immunoglobulin (particularly IgE) responder rat strain. In some ways the Brown Norway rat resembles atopic humans in their genetic predisposition to develop allergies. Preliminary experiments whereby ovalbumin was used as the sensitizing antigen, demonstrated that the BN rat is a promising species for the development of an oral sensitization animal model. Furthermore, using immunoblotting experiments it was demonstrated that sera from BN rats which were sensitized orally with hen's egg white (HEW) and cow's milk (CM) and sera of allergic patients to hen's egg white or cow's milk recognized a comparable profile of allergens in these allergenic food products. This indicates that the specific protein recognition of induced antibodies in the BN rat is comparable to that observed in sera from allergic patients (detailed by Knippels et al. in this monograph).

In addition to their usefulness in systemic sensitization research, the BN rat and the BALB/c mouse models exhibit many of the characteristics of allergic asthma observed in humans i.e. immediate bronchial hyperresponsiveness and increased levels of IgE antibodies in serum and bronchoalveolar lavage fluid upon exposure to respiratory allergens such as dust mite allergens and molds. One present limitation of these models is that, although many asthma symptoms are exhibited, the animals do not go on to develop chronic respiratory disease, as is the case with human asthma. Eventually the rodents become tolerant to the allergen. A number of possibilities are being explored to overcome this limitation, such as circumventing the potential for tolerance by exposing the animals when very young.

The HLA class II transgenic mouse was shown to be an excellent model for studying the genetic and molecular basis of allergic hyperresponsiveness because, like humans, this mouse strain develops pulmonary eosinophilia, lung tissue damage, and airway hyperreactivity upon exposure to allergens. This was demonstrated by exposing the animals to extracts of short and giant ragweed. T-cell epitopes were identified and HLA haplotype DQ transgenic mice exhibited strong T-cell responses to short ragweed extracts. It was shown that only the HLA-DQ mice exhibited these responses, indicating that a response-specificity exists for different HLA molecules. It was also shown that this response was mediated by CD4+ T-cells. This model holds promise for identifying epitopes critical in eliciting allergic responses as well as for the development of potential immunotherapies (see paper in this monograph by Chapoval and Chella).

A functional *in vitro* assay that predicts T-cell responses to peptide epitopes in humans was also presented (see paper by Stickler et al., in this monograph). It was specifically developed to predict functional T-cell epitopes in individuals who had not been previously exposed to the

protein in question. For the purposes of the allergenic assessment of GM-proteins, this is a very important consideration since previous human exposure to most novel recombinant proteins has obviously never occurred. Determining the T-cell epitopes of potentially allergenic substances may also aid in developing variants which may be hypo-allergenic, thus reducing the overall allergenic potential of GM-proteins and other novel substances.

Although there are practical considerations which make rodents the model of choice for many labs, several non-rodent models have proven useful in evaluating food allergenicity. The swine model and the atopic dog model were described at the workshop. The advantage of both models is in their propensity to develop clinical symptoms of food allergy, primarily gastrointestinal and dermatological reactions, after the sensitized animal is challenged with food antigens. In contrast, clinical responses to food allergens in rodent models are not as reminiscent of human responses.

Swine are frequently used in research as a surrogate for humans. Developing piglets have anatomic and nutritional similarities to developing humans, including a tendency to become either sensitive or tolerant to soy and cow's milk proteins. In the swine model of food allergenicity, newborn piglets are sensitized to peanut protein by intraperitoneal injection of peanut extract plus the adjuvant cholera, followed by two subsequent booster injections 18 and 25 days later. Oral challenge of sensitized piglets results in gastrointestinal and dermatologic symptoms which can be measured using direct skin testing to elicit wheal and flare reactions or by assessing changes in gut morphology including edema and hemorrhage. A limitation of this model, which will undoubtedly be overcome in the near future, is the lack of antibodies specific for swine IgE. As a result the presence of food antigen-specific IgE in sensitized animals has not yet been confirmed.

Food allergy is relatively common in dogs, affecting about 8% of the canine population. The atopic dog model is based on an inbred colony of high IgE-producing dogs. This model has the advantage that gastrointestinal and dermatologic responses to food allergen challenge in sensitized animals have been correlated with increased circulating antigen-specific IgE. To elicit sensitization, dogs are immunized with live virus vaccine, followed by several subcutaneous injections with food antigens over a course of weeks. At this point the dogs produce antigen-specific IgE, respond with wheal and flare reactions in skin tests, and show signs of gastrointestinal edema and inflammation. The applicability of the atopic dog model has been proven in a study of the potential allergenicity of a genetically modified corn line. Both transgenic and non-transgenic corn leaf extracts were found to be essentially non-allergenic compared to common food allergens in pups sensitized from birth with either of the two leaf protein extracts and with common food allergens such as peanut, soy and cow's milk. These data indicate that non-rodent models should not be overlooked as a source of valuable data on the potential allergenicity of genetically modified foods.

In summary, a number of rodent and non-rodent animal models of respiratory and systemic sensitization with allergens are currently been evaluated for their potential to predict allergenicity to food, plant and animal substances. While there is presently no single animal

model which meets the requirements for an ideal animal model, each of these models have merits which, when further validated, can contribute significantly to the overall assessment of allergenicity to several substances including the GM-derived protein products. The development of *in vitro* assays to predict functional T-cell epitopes in individuals who had not been previously exposed to the culprit proteins can be a very powerful tool in the study of allergenicity to substances. Perhaps the most useful application of this novel technique would be the development of hypo-allergenic substance variants. This would help reduce the overall allergenic potential of GM-proteins and other novel substances.

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APPENDIX IV. DIETARY PHYTOESTROGENS; SAFETY, NUTRITIONAL QUALITY AND HEALTH CONSIDERATIONS

G. Sarwar Gilani, Ph.D.
Nutrition Research Division, Food Directorate, Health Canada

Possible health benefits and potential adverse effects of dietary phytoestrogens will be briefly reviewed. Data from two rat studies (a 16-week study with Sprague-Dawley rats and a life span study with Stroke-Prone Spontaneously Hypertensive rats) conducted at Health Canada laboratories to assess safety of soy isoflavones will be presented. Moreover, future planned research related to safety assessment of dietary phytoestrogens will be highlighted.

Study in Sprague-Dawley Rats:

In a 16-week study with weanling rats, the effects of the addition of graded levels (0, 50, 100, 200 and 400 mg/kg diet) of soybean isoflavones to a casein control diet on growth, plasma total cholesterol, plasma isoflavones and length of estrus cycle were investigated. An isoflavone-rich extract (Novasoy) and a soy infant formula were used as a source of dietary isoflavones. According to the initial analyses, there was a dose-related increase in the levels of plasma isoflavones. The plasma data showed that the absorption of isoflavones from soy formula was lower than that from Novasoy. There was also a dose-related increase in the length of the estrus cycle in female rats. The cycle in rats fed the formula diet was, however, shorter than that in those fed the Novasoy diet providing the same amount of isoflavones. This suggested differences in the potency based on the source of isoflavones.

Study in Stroke-Prone Spontaneously Hypertensive rats:

In recent studies the life span of Stroke-Prone Spontaneously Hypertensive (SHRSP) rats, one of the most suitable animal models for stroke in humans, was altered by the percentage of dietary protein. Death due to stroke occurred significantly earlier in animals fed 10% protein casein diet compared to those fed 20 and 40% protein casein diets. This study was conducted to examine whether source of dietary protein (casein v. soybean protein isolate, alcohol washed to remove most isoflavones), type of supplemental sulfur amino acid (methionine v. cystine), dietary isoflavones and anthocyanins modulate the life span of SHRSP rats. Body weight and systolic blood-pressure matched groups of 47 day-old SHRSP rats received semi-purified diets containing 200 g/kg protein (casein or soybean) supplemented with methionine or cystine (3 g/kg), 0 or 500 mg/kg isoflavones (from Novasoy), and 0 or 500 mg/kg anthocyanins (extracted from grapes). All the experimental diets also contained 10% soybean oil and required levels of other nutrients. A 0.5% sodium chloride solution was used as drinking water to induce hypertension. After consuming the diets for 37 days, ten animals from each dietary group were killed for the collection of blood and tissues for biochemical analyses which are currently underway. The death rates of rats fed the casein and soybean protein diets were not different. Death due to stroke was significantly earlier ($P < 0.0001$) in the animals fed cystine-supplemented diets compared to those fed methionine-supplemented diets. Addition of

isoflavones or anthocyanins had a significant negative effect ($P < 0.023$, $P < 0.016$) on the survival rates of rats.

Future Planned Research (2002-2003):

1. Development of improved HPLC-mass spectrometry methods for the analysis (concentration and composition) of isoflavones in biological fluids and tissues.

2. Analyses of tissues from the 16-week rat study and from the Health Canada multi-generation study to suggest upper safe limits of dietary isoflavones.

3. Further assessment of the source (endogenous vs. extracted) of isoflavones on their bioavailability and bioactivity.

4. Influence of dietary proteins (varying in isoflavones and methionine) on the incidence of stroke in SHRSP rats.

APPENDIX V. TECHNICAL DISCUSSION PROGRAMME

PROGRAMME

Tuesday April 30, 2002 (8:00 - 5:00)
Chateau Cartier Resort
Gatineau, Quebec

Introduction

In November 2001, the Government of Canada published an action plan in response to the Expert Scientific Panel of the Royal Society of Canada report entitled: *Elements of Precaution: Recommendations for the Regulation of Food Biotechnology in Canada*. The action plan describes specific actions and projects that departments intend to carry out in response to the Expert Panel's recommendations. In this action plan, departments also commit to collaborate with external experts to further enhance many areas of the regulatory process and protocols. To encourage further technical dialogue and advancement of the technical knowledge in areas related to the regulation of GM-foods, Health Canada is holding a technical discussion focussed on the health and safety aspects of the action plan.

The objective of this meeting will be to seek input on how to further develop the projects identified in the action plan dealing with the health and safety of GM-foods, as well as identify new activities to be initiated, ensuring the continued technical contributions and collaboration of external experts with the Government of Canada.

8:00 **Registration** (50 min.)

8:50 **Welcome and Opening Remarks** (10 min.)
Dr. Karen Dodds, Director General, Food Directorate, Health Canada

9:00 **Overview of Codex Guidelines for the Safety Assessment of Recombinant-DNA Plants** (10 min.)
Mr. Paul Mayers, Acting Associate Director General, Food Directorate, Health Canada

Health Canada's Initiatives Regarding the Health and Safety Aspects of the Action Plan

Presentations:

9:10 **Molecular Characterization Used in the Safety Assessment of Novel Foods**
Dr. William Yan, Chef, Evaluation Division, Bureau of Microbial Hazards

9:25 **DNA Microarray Technology: Applications and Uses in the GM-Area**
Dr. Franco Pagotto, Research Scientist, Bureau of Microbial Hazards

9:40 Toxicology and Allergenicity - Overview of Current and Future Research
Dr. Rekha Mehta, A/Chief, Toxicology Research Division, Bureau of Chemical Safety

9:55 Dietary Phytoestrogens: Safety, Nutritional Quality & Health Considerations
Dr. Ghulam Sarwar Gilani, Senior Research Scientist, Bureau of Nutritional Sciences

10:10 Update on the Biotechnology Surveillance Project
Rita Beregszaszy, Project Manager, Centre for Surveillance Coordination

10:15 Health Break (15 min.)

10:30 Breakout Session A (2h)
Participants may attend one of the following breakout groups:
-Molecular Characterization
-Allergenicity
-Nutrition

12:30 Lunch (1h)

1:30 Breakout Session B (2h)
Participants may attend one of the following breakout groups:
-Allergenicity
-Toxicology
-Long-term surveillance

3:30 Health Break (15 min.)

3:45 Next steps (1h)

4:45 Concluding Remarks (15min.)