CANADA AND UNITED STATES
BILATERAL ON AGRICULTURAL BIOTECHNOLOGY

APPENDIX II:
ENVIRONMENTAL CHARACTERIZATION DATA FOR TRANSGENIC PLANTS INTENDED FOR UNCONFINED RELEASE

INTRODUCTION

Representatives of the Canadian Food Inspection Agency, Plant Biosafety Office; the United States Department of Agriculture, Animal and Plant Health Inspection Service; and the United States Environmental Protection Agency, Office of Pesticides Program met on September 20 and 21, 2000 in Riverdale, Maryland to discuss components considered in environmental risk assessments for genetically engineered plants. The working model for the project is the 1998 Canada-USDA-APHIS document on Molecular Genetic Characterization Data (MGCD). The environmental data are seen as a complement to the molecular characterization data which Canada and the United States identified in their technical discussions in 1998 (http://www.cfia-acia.agr.ca/english/plaveg/pbo/usda02e.shtml). The main objective of the meeting was to identify the elements of the assessment. The outcome of the project will be a living document (subject to revision as necessary) that identifies both the commonalities and the differences in the environmental data which are used for scientific assessment of transgenic plants before they are authorized for unconfined environmental release. Considerations specific to confined field testing were beyond the scope of this project.

Information in this appendix is used to evaluate the differences between the way the transgenic plants and their nontransgenic counterpart interact with the environment in managed and unmanaged ecosystems. Such information is useful in assessing the likelihood that the plant will be harmful to the environment, either directly or indirectly. Agreement on common data needs and acceptable analytical approaches for these evaluations will assist in the submission of data by developers seeking regulatory approval to introduce these plants into agricultural production or commerce. Specific information may vary with plant species, the specific types of modifications, and end use. These information criteria have been developed mainly for crop plants and may not include information criteria for trees and aquatic plants. For some plants, based on their biology, certain information may not be appropriate. In addition to these criteria, the participants of both countries reaffirmed that reviews are still conducted on a case-by-case basis which allows for reviewing additional or fewer criteria, depending upon the individual case and the regulatory authority of the individual agencies.

GLOSSARY

Biodiversity - The variability among living organisms from all sources including, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and between ecosystems.

Confined Field Testing - Release of transgenic plants into the environment under terms and conditions intended to minimize establishment and spread into, and interaction with the environment of the transgenic plant and any progeny derived from it.

Counterpart - The plant which represents the closest available approved genotype to the transgenic plant in question and is a suitable control. In some instances this may be a transgenic progenitor plant.

Cultivate - To produce or grow in an agricultural system.

Environment - Components of the earth (including air, land, water, all layers of the atmosphere, all organic and inorganic matter and living organisms) and all interacting natural systems. This includes both unmanaged and managed ecosystems.

Genotype - The sum total of the genes of an organism, latent or expressed.

Irritant - Any agent capable of eliciting an abnormally excited or sensitive condition in a body part of a human or other animal.

Microfauna - Microscopic animals.

Microflora - Microscopic plants, bacteria and fungi.

OECD Consensus Documents - Reports published by the Organisation for Economic Cooperation and Development (OECD) that contain technical information for use in the regulatory assessment of products of biotechnology. The documents are mutually recognized among member countries of the OECD.

Outcrossing - Sexual reproduction involving cross fertilization with other plant genotypes.

Outcrossing frequency - The percentage of total progeny produced by a plant as a result of outcrossing.

Parthenocarpy - Production of fruit without fertilization.

Phenotype - The observable characteristics of an organism (including physical, biochemical or other traits) which may result from the interaction of the organism with it's environment.

Threatened and Endangered Species - Organisms listed as such by Federal authorities: the U.S. Departments of Interior or Commerce who jointly administer under the authority of the Federal Endangered species Act; in Canada the proposed Species At Risk Act (SARA) will be administered by Environment Canada.

Transgenic Plant - A plant in which one or more genes, genetic constructs, or traits have been introduced using recombinant DNA techniques, which could be considered to include the insertion of genetic material from the same or different species.

Unconfined Release - A release into an environment of a transgenic plant that is not isolated either reproductively or physically from managed or unmanaged environments, but may be subject to other restrictions.

Unmanaged Ecosystem - A non-agricultural area not subject to significant human manipulation such as mowing, pesticide application, planting, etc.

INFORMATION FOR ENVIRONMENTAL ASSESSMENTS

1. DESCRIPTION OF THE BIOLOGY OF THE PLANT SPECIES PRIOR TO MODIFICATION

1.1 Provide common name(s) and currently accepted scientific nomenclature.

1.2 Describe the biology of the counterpart.

The U.S. and Canada each required information on the biology of the plant species which has been modified. The information requirement may be fulfilled completely or in part by reference to an appropriate biology document submitted to CFIA (Canada submissions only). Appropriate OECD Consensus Documents may partially or completely fulfill these requirements if such documents are available.

2. PHENOTYPE OF THE TRANSGENIC PLANT

The applicant must provide information on the intended phenotype and any unintended or unanticipated traits. The transgenic plant should be compared to its counterpart(s), and related cultivated varieties as appropriate. If changes are detected, the applicant should address these findings in the submission.

Typically, observations are made when the plants are grown in multiple sites and over more than one growing season. The testing locations should be representative of the normal growing regions of the crop. In some cases, such as where there may be a potential for increased weed characteristics or if the crop is an outcrossing species, it may be appropriate to evaluate the plants outside of managed ecosystems. Depending upon the results, additional studies may be warranted to provide the additional information. In some cases, applicants may provide valid scientific rationale why certain information is unnecessary or inappropriate to provide.

2.1 Describe the breeding history of the transgenic plant population being evaluated starting at the point of trait introduction.

2.2 Compare the transgenic plant to its counterpart(s) with respect to the following characteristics which influence reproductive and survival biology:

a. **Growth habit** - e.g., Note any changes in basic morphology of the plant including any abnormalities.

b. **Life-span** - e.g., Plants can be categorized as annual, biennial, perennial. Would the presence of the introduced trait produce a change?

c. **Vegetative vigor** - e.g., plant height, crop biomass, etc.

d. **Ability to overwinter (or overseason)**

e. **Number of days to onset of flowering; number of days for flowering**

f. **Number of days until maturity** - Depending on the plant species, this could be defined as the time to the production of mature fruit or seed (suitable for harvesting). In many species this characteristic is dependent upon factors such as day length and/or degree days.

g. **Seed parameters**

Seed production - This could be measured as either yield (number of seeds or fruit per cultivated area) or the number of viable seeds per plant.

Continuous seed/ fruit production - Length of time (days) of seed/fruit production. This might include but is not limited to changes between determinant and indeterminant flowering.

Seed dormancy - For example, characterize any changes in the ability of the seed to remain viable over time.

Seedling emergence - Proportion of seeds planted that emerge as seedlings under field conditions and a description of the various environmental conditions, to evaluate emergence in more variable environments, especially those of unmanaged ecosystems.

h. **Proportion surviving from seedling to reproduction**

i. **Outcrossing frequency**

j. **Impact on pollinator species** - This might be addressed through information on whether the same pollinator species have been seen in the field or have there been changes in pollinator species visiting the flowers. Data on changes in flower morphology, color, fragrance, etc. might also indicate interactions with pollinators may have been altered.

k. **Pollen parameters**

i) Amount of pollen produced, proportion of viable pollen, the longevity of pollen under varying environmental conditions.

ii) Physical parameters such as stickiness, shape, and weight that might affect the viability or performance of the pollen in leading to successful pollination.

l. **Fertility** - consider whether the plants have acquired or lost fertility.

m. **Self-compatibility**

n. **Asexual reproduction**, i.e. vegetative reproduction; ability of the plant material to set roots; parthenocarpy

o. **Seed dispersal factors** - This might be addressed by considering characteristics such as seed shattering or dispersal by animals.

p. **Symbionts** - e.g., vesicular-arbuscular mycorrhizal fungi, rhizobia.

q. **Stress adaptations** (Specifically note which stresses were observed):

i) **Biotic stress factors**: Examples might include parasites or pathogens, competitors (e.g.,weeds), and herbivores.

ii) **Abiotic stress factors**: Examples might include response to moisture stress, nutrient deficiency, or other stresses common to that species.

2.3 Compare the compositional analysis of the transgenic plant to its counterpart(s) including, protein, lipids, fiber, and other parameters as appropriate. This data is used to assess secondary or pleiotropic effects and may indicate environmental impacts (e.g., changes in nutritional quality of seeds affecting birds).

2.4 Compare the transgenic plant and its counterpart(s) with respect to levels of known naturally expressed toxicants and antinutrients known for that species.

3. CULTIVATION OF THE TRANSGENIC PLANT

3.1 Description of intended cultivation area

3.1.1 Describe the regions where the plant will be grown. This might include information on whether the intent is to cultivate the transgenic plant in all parts of the country or only in specific regions. How do the projected areas of cultivation compare with the usual managed ecosystems for the species? Is there likely to be a change in the total projected area of cultivation?

3.1.2 If the new trangenic plant will be cultivated in areas outside its normal geographic areas, then identify and describe the new ecosystems in which the transgenic plant will be cultivated.

3.2 Description of cultivation practices

3.2.1 Describe the cultivation practices for the transgenic plant, including land preparation, fertilizer usage, weed and pest control, harvest, post-harvest protocols, and other cultivation practices. Compare and contrast these practices from those traditionally used for this species. Discuss how such practices might influence agro-ecosystem sustainability, crop rotations, pesticide use, frequency of tillage, soil erosion and consequential changes in energy and soil conservation. Discuss in what ways any volunteer plants of the transgenic plant may dictate altered management practices for succeeding crops?

3.2.2 Describe any specific deployment strategies recommended for this transgenic plant? Deployment strategies might include geographic or temporal factors or integration with other practices.

3.2.2.1 Insect Resistance Management - In the case of insect resistant transgenic plants, describe strategies intended to delay the development of resistance in target insect populations.

3.2.2.2 Herbicide Resistant Crop Management - In the case of transgenic plants developed for resistance (tolerance) to a herbicide or class of herbicides, describe appropriate strategies that are intended to delay the development of herbicide resistant weeds and avoid significant changes in weed biotypes.

4. INTERACTIONS OF THE TRANSGENIC PLANT WITH SEXUALLY COMPATIBLE SPECIES

Determine whether there are any sexually compatible species in areas where the transgenic plants will be grown. If there are, then this section is applicable and the following questions should be considered.

4.1 Which sexually compatible species, if any, are found in areas where the crop will be cultivated, including any new areas of cultivation?

4.2 Characterize the compatible wild relative(s) with respect to weediness in managed ecosystems and/or establishment and spread into unmanaged ecosystems

4.3 In what ways would the introduced trait itself be likely to change the ability of the transgenic

plant to interbreed with other plant species?

4.4 In cases in which there is a potential for gene flow from the transgenic plant into sexually compatible species (e.g. same or related species as appropriate), describe the consequences for the offspring of such crosses. Characterization of the crosses between wild relatives and transgenic plants should be considered using the criteria described in section 1 for transgenic plants in order to address questions 4.4.1 and 4.4.2 below.

4.4.1 Is the introduced trait similar to a trait found currently in natural populations of the compatible wild relatives?

4.4.2 Does the introduced trait have the potential to increase the reproductive fitness or confer a selective advantage on the wild relative? If so would the introduced trait have a significant impact on the establishment and spread of populations of wild relatives? Consider the presence or absence of selection pressures.

4.4.2.1 Is the potential for the trait to increase reproductive fitness or confer a selective advantage different than the potential for this to occur from a similar trait that may already exist for the same crop?

5. RESIDUAL EFFECTS AND TOXICITY ON NONTARGET ORGANISMS

5.1 Characterize the extent to which the gene product has been a part of the human or animal diet.

5.2 Characterize to what extent the introduced DNA directly or indirectly leads to the expression of a toxin or other product that is known to affect metabolism, growth, development, or reproduction of animals, plants, or microbes?

5.3 Consider potential physiological and behavioral effects to other organisms including insect, avian, aquatic, or mammalian species in the areas where the crop will be cultivated, including any new area of cultivation.

Consideration may be given to:

- threatened and endangered species in the area where the crop is to be grown.

- beneficial organisms (pollinators, predators, parasites, biological control organisms, soil microbes)
- other appropriate nontarget organisms (Canada in all cases, U.S. in the case of plant incorporated protectants).

Consider levels and routes of exposure to all plant parts that express the gene, i.e., direct feeding or other exposure to the plant or plant part, dispersed plant parts, secretion, degradation, or leaching of the active toxic component, gene introgression (see 5.3), or organisms that have fed on the plant. The molecular genetic characterization section may be useful in analyzing the routes of exposure because it includes information on expression of the gene product(s) in various plant tissues.

The residual effects of the transgenic plant in comparison to nontransgenic plants may be assessed by crop rotation studies (see DIR94-08) [Canada only] or other techniques.

5.4 Characterize potential adverse effects on the health of humans (including workers, adults, and children) which may arise through physical contact with or use of the transgenic plant or its parts or its raw or processed products, other than for uses for which other authorizations or reviews are required (e.g. food, feed, pharmaceuticals). The analysis might include a comparison of the transgenic and non-transgenic counterpart(s) with respect to the likely exposure to toxins, irritants, and allergens.

6. OTHER ENVIRONMENTAL INTERACTIONS

6.1 In the case of transgenic plants developed using plant viral coding regions, address synergy, facilitated movement, transcapsidation, and viral recombination. See the OECD consensus document for a description of these terms.