PN-ACS-447

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Ministry of Agriculture & Land Reclamation
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Agriculture Policy Reform Program
Reform Design and Implementation

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Ministry of Agriculture and Land Reclamation

AGRICULTURE POLICY REFORM PROGRAM

Reform Design and Implementation Unit (RDI)

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Reform Design and Implementation Unit

Development Alternatives Inc. Group: Office for Studies & Finance, National Consulting Firm Development Associates, Cargill Technical Services, The Services Group, Training Resources Group, Purdue Universities, University of Maryland

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Report No. 139

Regulating the Commercialization of Transgenic Plants in Egypt: Environmental Safety Assessment

Prepared Upon The Request Of:

The Executive Secretary
of the National Biosafety Committee
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By

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With

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June 2001

B

Contents

Part 1: Overview and Organization of the Report

Part 2: Proposed Section III of the Biosafety Regulations and Guidelines: Procedure for Commercial Approval of Genetically Modified Plants in Egypt.

Part 3: Proposed Appendix IX to the Biosafety Regulations and Guidelines: Guidelines for the Environmental Safety Assessment of Genetically Engineered Plants

Part 4: Risk Assessment Principles Provided by the Cartagena Biosafety Protocol of the Convention on Biodiversity.

Part 5: Annex IIIB of the Proposed European Directive: Information Required in Notifications Concerning Releases of Genetically Modified Higher Plants (GMHPs) (Gymnospermae and Angiospermae)

Part 6: Requirements for a Petition for Determination of Nonregulated Status from USDA/APHIS

Part 7: Information Required by the Canadian Food Inspection Agency, Plant Biosafety Office, for Determining Environmental Safety Prior to Approval of Unconfined Release

Part 8: Consistency of the Information Requirements of Selected Regions or Countries with the Risk Assessment Points to Consider Provided In Annex III of the Cartagena Biosafety Protocol of the Convention On Biodiversity

Part 9: Application of Environmental Safety Guidelines to the Environmental Safety Assessment of Potato Variety "Spunta" Genetically Engineered to be Resistant to Potato Tuber Moth

Annex 1: Resource Documents on the Biology of Potato from OECD, CFIA, and USDA

Annex 2: Joint USDA/CFIA molecular characterization and data standards.

Annex 3: *Powerpoint* Presentation at Workshop of Experts Gathered at AGERI on June 20, 2001

Annex 4: Preliminary Report on Existing Facilities and Human Resource Capacity for Evaluation of Environmental Safety of Transgenic Plants in Egypt

C

Part 1. Overview and Organization of the Report

Scientists in both the public and private sectors in Egypt are currently using the techniques of genetic engineering to develop new, improved varieties of crops for eventual use by Egyptian farmers. Up until now, this work has been restricted to research and contained testing, but soon some new varieties will become ready for commercial release. Such release will need to be regulated to ensure safety.

Egypt's National Bio-Safety Committee (NBC) uses the Biosafety Regulations and Guidelines to guide its work in regulating the use of genetically engineered plants in Egypt. These Guidelines focus mainly on issues of controlled testing. The Guidelines are underdeveloped when it comes to specifying requirements for approving the release of such plants for commercial purposes. This report proposes a draft amendment to the Guidelines (see Part 2) to remedy this deficiency, with particular emphasis on the necessity of : (a) food and feed safety assessments, and (b) environmental safety assessments, as conditions for approval.

An earlier report (Quemada et al., RDI Report #134, 2001) proposed detailed guidelines for the food and feed safety assessment of foods derived from genetically engineered plants. These guidelines described the data and test results to be supplied by the applicant to the NBC, including (1) characteristics of the genetically engineered plant and the effects of the transformation on known toxicants, antinutrients, or nutrients, (2) the sources of new genetic materials and their characterization in the genetically engineered plant, and (3) safety assessment of the gene products in the genetically engineered plant.

The current report proposes similar guidelines for the assessment of environmental safety of transgenic plants (see Part 3). These guidelines describe the data and test results to be supplied by the applicant to the NBC, including information on the recipient plant, the donor organisms and vectors, and the genetically engineered plant and its effect on the receiving environment. Issues addressed include (1) weediness, (2) effects on non-target organisms, and (3) relevant information on biological diversity and centers of origin of the likely receiving environment.

As a party to the Convention on Biological Diversity (CBD), Egypt has also signed the Convention's Cartagena Protocol on Biosafety.[1] The environmental safety assessment guidelines proposed in this report are based on a review of the Cartegena Protocol and existing risk assessment guidelines from the European Union and other countries (see Parts 4-8). The proposed guidelines are consistent with the Cartagena Protocol, and they incorporate the most important and specific data requirements used in other countries' risk assessment regulations.

The study team examined the capablities of Egyptian laboratories to conduct the tests needed to generate the data required for envrionmental safety assessments. This examination suggests that local laboratories can carry out the necessary testing either on behalf of the applicant or on behalf of the NBC. Annex 4 indicates which laboratories are best suited to do the various tests.

[1] At this point, 103 countries have signed the Convention, but only four (Bulgaria, Fiji, Norway, and Trinidad and Tobago) have ratified it (http://www.biodiv.org/world/parties.asp?lg=0)

It is up to the NBC to consider these proposed amendments to the Biosafety Regulations and Guidelines, adapt them to Egyptian needs, and arrange for their official approval. Once the guidelines are finalized and adopted, the NBC will be able to assess the food and environmental safety of new genetically-engineered plants proposed for commercial release in Egypt, and, when appropriate, recommend their registration to the Variety Registration Committee.

This report is organized as follows:

Part 2: Proposed Section III of the Biosafety Regulations and Guidelines: Procedure for Commercial Approval of Genetically Modified Plants in Egypt.

As pointed out in the previous report, the NBC should consider adding an additional section to the body of its guidelines to specify the procedures for granting commercial approvals. This updated version of the proposed section adds a new option to the committee under articles 4 and 5 – "to approve the application with additional conditions imposed." This option will allow the NBC to approve applications while requiring the applicant to restrict the size or location of its commercialization of the plant during an initial specified period, if that is deemed appropriate, or impose additional reporting requirements.

Part 3: Proposed Appendix IX to the Biosafety Regulations and Guidelines: Guidelines for the Environmental Safety Assessment of Genetically Engineered Plants

These are the heart of the report – the actual proposed guidelines. After adapting them to meet its needs, the NBC should consider adding these guidelines as an appendix to its Biosafety Regulations and Guidelines then use them to guide its risk assessement review work. The guidelines, of course, should also be made available to applicants to guide their own data collection work in consultation with the NBC. Information responsive to these guidelines may be obtained from the existing literature, observational data gathered from cultivated or natural populations, or specific tests. On a case by case basis, the National Biosafety Committee can specify the applicable data requirements and types of data adequate to answer each data requirement.

Part 4: Risk Assessment Principles Provided by the Cartagena Biosafety Protocol of the Convention on Biodiversity

This is an exerpt from the Biosafety Protocol. The proposed Guidelines for the Environmental Safety Assessment of Foods Derived from Genetically Engineered Plants are fully consistent with the Cartagena principles.

Part 5: Annex IIIB of the Proposed European Directive: Information Required In Notifications Concerning Releases of Genetically Modified Higher Plants (GMHPs) (Gymnospermae and Angiospermae)

This is the relevant part of the European guidelines for risk assessment.

Part 6: Requirements for a Petition for Determination of Nonregulated Status From USDA/APHIS

This is the relevant part of the U.S. guidelines for risk assessment.

Part 7: Information Required by the Canadian Food Inspection Agency, Plant Biosafety Office, for Determining Environmental Safety Prior to Approval of Unconfined Release.

This is the relevant part of the Canadian guidelines for risk assessment.

Part 8: Consistency Of the Information Requirements of Selected Regions or Countries with the Risk Assessment Points to Consider Provided In Annex III Of the Cartagena Biosafety Protocol Of The Convention On Biodiversity.

This section compares the information requirements set by the European, U.S., and Canadian guidelines by section, e.g. information relating to the recipient plants, donor organism, vector, insert, living modified organism, receiving environment, etc.

Part 9: Application of Environmental Safety Guidelines to the Environmental Safety Assessment of Potato Variety "Spunta" Genetically Engineered to be Resistant to Potato Tuber Moth

This part provides a practical illustration of the application of the proposed Egyptian guidelines to the environmental risk assessment of a potato genetically engineered for resistance to potato tuber month. It complements a similar application presented in the previous report, which applied the proposed guidelines for food and feed safety assessment to this potato.

Annex 1 contains resource documents on the biology of potato from OECD, CFIA, and USDA.

Annex 2 presents joint USDA/CFIA molecular characterization and data standards.

Annex 3 is the *Powerpoint* presentation made at a workshop of experts held at AGERI on June 20, 2001 to present and discuss the consultants' findings.

Annex 4 is a preliminary assessment of the existing facilities and human resource capacities for the evaluation of environmental safety of transgenic plants in Egypt, prepared by Prof. Dr. Amin Sayed El-Nawawy.

Annex 5 is a review of current decrees and laws impacting on the commercial release of transgenic plants, including a diagram outlining steps for release prepared by Prof. Dr. Amin Sayed El-Nawawy.

References

El-Badry, E., El-Tayeb, O. 1999. Report and Proceedings of Activities for Preparation of National Framework on Biosafety in Biotechnology. National Biodiversity Unit, Department of Nature Protection, Egyptian Environmental Affairs Agency, Ministry of State for Environment Affairs.

Madkour, M., El Nawawy, A., Traynor, P., 2000. Analysis of a National Biosafety System: Regulatory Policies and Procedures in Egypt. International Service for National Agricultural Research, ISNAR Country Report 62.

Ministry of State for Environmental Affairs, 1997. Egypt: First National Report to the Convention on Biological Diversity. Ministry of State for Foreign Affairs, Egyptian Environmental Affairs Agency, National Biodiversity Unit.

Quemada, H., Kent, L., and Magnani, R., 2001. Regulating the Commercialization of Transgenic Plants for Food and Feed in Egypt: Food Safety Assessment. Report No. 134 of the Reform Design and Implementation Unit, Ministry of Agriculture and Land Reclamation/US Agency for International Development/Agriculture Policy and Reform Program.

United Nations Environment Programme, 1996. International Technical Guidelines for Safety in Biotechnology.

Part 2

Proposed Section III of Biosafety Regulations and Guidelines: Procedure for the Commercial Approval of Genetically Modified Plants in Egypt

The applicant is required to make available to the National Biosafety Committee the information and data described below. The NBC will review and assess this information to inform its decision on the application.

1. Prior to submission of an application for registration of a genetically engineered variety to the Variety Registration Committee, the applicant must:

    a. Consult with the National Biosafety Committee Regarding the food and environmental safety assessments that must be conducted. The National Biosafety Committee will make recommendations with the advice and consent of the Supreme Committee for Food Safety. For foreign applicants, the NBC can decide whether to accept results of tests accepted and approved by regulatory agencies of other countries.
    b. Arrange for the testing and collection of data required by the National Biosafety Committee.
    c. Obtain the approval of the National Biosafety Committee, which will be forwarded to the Variety Registration Committee.

2. The information submitted for assessment of food safety should be consistent with the guidelines set forth in Appendix VIII.

3. The information submitted for assessment of environmental safety shall be consistent with the guidelines set forth in Appendix IX.

4. The National Biosafety Committee and the Supreme Committee on Food Safety, will review the information submitted, and decide:

    a. To approve the application,
    b. To approve the application with additional conditions imposed, or
    c. Require further tests and consultations where additional requested data are reviewed. Additional tests may be requested especially when the results of the first assessment reveal a safety concern that needs to be resolved.

5. Further consultations shall take place until the following endpoints are reached:

    a. The National Biosafety Committee approves the application, with concurrence by the Supreme Committee for Food Safety;

b. The National Biosafety Committee approves the application, with concurrence by the Supreme Committee for Food Safety, with additional conditions imposed;
c. The National Biosafety Committee rejects the application because the plant is judged to be unsafe;
d. The National Biosafety Committee and/or the applicant conclude that remaining safety issues cannot be resolved scientifically; or
e. The applicant withdraws the application.

6. The applicant may resume the application for approval, at which time the process for approval begins at the point at which it was terminated.

7. Upon approval, the National Biosafety Committee forwards its decision and recommendation to the Variety Release Committee in accordance with MALR Decree 1648/1998.

8. The National Biosafety Committee may call on experts in relevant scientific fields to assist in the review of the data presented, or to serve in a general advisory capacity during the assessment of specific applications.

9. The decisions of the National Biosafety Committee stating the basis for their decision shall be published in printed form, and made accessible via the internet or other publicly available media that the Minister of Agriculture deems appropriate.

10. After approval, the National Biosafety Committee will forward a copy of the applicant's information package to laboratories that require it to develop the necessary testing to detect transgenic material if mandated by any future labelling or quality standards regulations.

Part 3

Proposed Appendix IX to the Biosafety Regulations and Guidelines: Guidelines for the ENVIRONMENTAL Safety Assessment of Genetically Engineered Plants

The following guidelines for data requirements and safety assessment are proposed[2]:

I. Information on the recipient plant or parental plants

A. Taxonomy:
   1. family name;
   2. genus;
   3. species;
   4. subspecies;
   5. cultivar/breeding line;
   6. common name.

B. Reproductive characteristics of the recipient plant
   1. Information concerning reproduction:
      a. mode(s) of reproduction;
      b. specific factors affecting reproduction, if any;
      c. generation time.
   2. Sexual compatibility with other cultivated or wild plant species, including the distribution in Egypt of compatible species.

C. Survivability:
   1. ability to form structures for survival or dormancy;
   2. specific factors affecting survivability, if any.

D. Allelopathy

E. Dormancy

F. Dissemination (pollen, seed, vegetative means):
   1. ways and extent (e.g. an estimation of how viable pollen and/or seeds declines with distance) of dissemination;
   2. specific factors affecting dissemination, if any.

G. Geographical distribution of the plant, including the centers of origin and genetic diversity, if known.

H. In the case of plant species not normally grown in Egypt, description of the natural habitat of the plant, including information on natural predators, parasites, competitors and symbionts.

I. Information on the location, geographical, climatic and ecological characteristics, including relevant information on biological diversity and centers of origin of the likely potential receiving environment

J. Other potential interactions of the plant with organisms in the ecosystem where it is usually grown, or elsewhere, including information on toxic effects on humans, animals and other organisms.

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[2] Information responsive to these guidelines may be obtained from the existing literature, observational data gathered from cultivated or natural populations, or specific tests. On a case by case basis, the National Biosafety Committee can specify the applicable data requirements and types of data adequate to answer each data requirement.

II. The sources of the new genetic materials (donor organisms and vectors), their characterization in the genetically engineered plant

A. Information on the donor organism(s) and, when appropriate, on other members closely related to the donor organism(s), including:
   1. its scientific name
   2. taxonomic classification
   3. usual or common name.

B. Information on all genetic material potentially delivered to the engineered plant:
   1. the specific method used for transformation
   2. the vector DNA, including the source (e.g. plant, microbial, viral, synthetic), identity and expected function in the plant
   3. intermediate host organisms including the organisms (e.g. bacteria) used to produce or process DNA for transformation of the host organism
   4. The DNA to be introduced (other than the vector), including:
      a. the characterization of all the genetic components including marker genes, regulatory and other elements affecting the function of the DNA
      b. the size and identity
      c. the location and orientation of the sequence in the final vector/construct
      d. the function

C. Molecular and biochemical characterization of the DNA actually inserted into the plant genome, including[3]:
   1. The number of insertion sites and location (e.g. chromosomal, chloroplast, mitochondrial
   2. The organisation of the inserted genetic material at each insertion site, including sequence data of the inserted material and of the surrounding region if deemed necessary. Information should be presented regarding the arrangement of the genetic material used for transformation has been conserved or whether significant rearrangements have occurred upon integration.
   3. The level and site of expression in the plant of the introduced gene product(s)[4],
   4. Data to demonstrate that all introduced traits are stably expressed and inherited.

[3] Additional guidance for molecular characterization is provided by a joint USDA/CFIA document (Summary Report , Canada - U.S. Bilateral Discussions on Agricultural Biotechnology, July 15-16, 1998 Ottawa, Ontario, CANADA Appendices I and Reviewers Checklists.) These documents are attached as Annex VIII, and can be used as guidance for the information required for food and feed safety assessment as well.

[4] This information is necessary not only for food and feed safety assessment, but also for the assessment of environmental questions such as the expected amount or residues of certain novel proteins. If the properties of those proteins present an environmental concern, then it would be appropriate to calculate the potential environmental exposure to them.

III. Information on the genetically engineered plant and its effect on the receiving environment

Information relating to known and potential differences between the genetically modified plant and the recipient plant in:

A. Plant pest risk characteristics
B. Disease and pest susceptibilities
C. Expression of the gene product
D. New enzymes
E. Changes to plant metabolism
F. Weediness of the genetically engineered plant, including:
    1. information concerning reproduction
        a. mode(s) or reproduction
        b. specific factors affecting reproduction, if any
        c. generation time
    2. dissemination (pollen, seed, vegetative means)
        a. ways and extent (e.g. an estimation of how viable pollen seeds declines with distance) of dissemination
        b. specific factor affecting dissemination, if any
    3. survivability
        a. ability to form structures for survival or dormancy
        b. specific factors affecting survivability, if any
    4. allelopathy
    5. dormancy
G. Impact on the weediness of any other plant with which it can interbreed
H. Intended use and agricultural or cultivation practices, including where applicable:
    1. altered geographic range of cultivation
    2. altered habitat adaptation
    3. altered cultivation practices, including
        a. information showing the effect of these changes on sustainability, especially with respect to pesticide use, frequency of tillage, soil erosion and consequential changes in energy and soil conservation
        b. information on any effect of volunteer plants on crop rotation practices
        c. specific containment or postharvest procedures, including procedures for disposal of remaining plant matter.
            i. any specified conditions for isolation as standard operating procedure for production
            ii. any specified conditions for handling crop rotations as standard operating procedure for production
            iii. any specified conditions for dealing with volunteers as standard operating procedure for production
        d. planting practices and policies (e.g. planting of mixtures to delay the evolution of resistance).

I. Effects of the genetically engineered plant on non-target organisms. If the introduced gene product is known to act by a toxic mechanism, the effects

on potential non-target organisms that might be affected by such a mechanism should be evaluated, including soil micro flora and fauna.

J. Indirect plant pest effects on other agricultural products
K. Transfer of genetic information to organisms with which it cannot interbreed
L. Potential interaction with the abiotic environment.
M. Description of detection and identification techniques for the genetically modified plant.
N. Information about previous releases of the genetically modified plant, if applicable, including past reports provided to other regulatory agencies.

Part 4

Risk Assessment Principles Provided by the Cartagena Biosafety Protocol of the Convention on Biodiversity

Objective

1. The objective of risk assessment, under this Protocol, is to identify and evaluate the potential adverse effects of living modified organisms on the conservation and sustainable use of biological diversity in the likely potential receiving environment, taking also into account risks to human health.

Use of risk assessment

2. Risk assessment is, inter alia, used by competent authorities to make informed decisions regarding living modified organisms.

General principles

3. Risk assessment should be carried out in a scientifically sound and transparent manner, and can take into account expert advice of, and guidelines developed by, relevant international organizations.

4. Lack of scientific knowledge or scientific consensus should not necessarily be interpreted as indicating a particular level of risk, an absence of risk, or an acceptable risk.

5. Risks associated with living modified organisms or products thereof, namely, processed materials that are of living modified organism origin, containing detectable novel combinations of replicable genetic material obtained through the use of modern biotechnology, should be considered in the context of the risks posed by the non-modified recipients or parental organisms in the likely potential receiving environment.

6. Risk assessment should be carried out on a case-by-case basis. The required information may vary in nature and level of detail from case to case, depending on the living modified organism concerned, its intended use and the likely potential receiving environment.

Methodology

7. The process of risk assessment may on the one hand give rise to a need for further information about specific subjects, which may be identified and requested during the assessment process, while on the other hand information on other subjects may not be relevant in some instances.

8. To fulfil its objective, risk assessment entails, as appropriate, the following steps:

(a) An identification of any novel genotypic and phenotypic characteristics associated with the living modified organism that may have adverse

effects on biological diversity in the likely potential receiving environment, taking also into account risks to human health;

(b) An evaluation of the likelihood of these adverse effects being realized, taking into account the level and kind of exposure of the likely potential receiving environment to the living modified organism;

(c) An evaluation of the consequences should these adverse effects be realized;

(d) An estimation of the overall risk posed by the living modified organism based on the evaluation of the likelihood and consequences of the identified adverse effects being realized;

(e) A recommendation as to whether or not the risks are acceptable or manageable, including, where necessary, identification of strategies to manage these risks; and

(f) Where there is uncertainty regarding the level of risk, it may be addressed by requesting further information on the specific issues of concern or by implementing appropriate risk management strategies and/or monitoring the living modified organism in the receiving environment.

Points to consider

9. Depending on the case, risk assessment takes into account the relevant technical and scientific details regarding the characteristics of the following subjects:

(a) Recipient organism or parental organisms. The biological characteristics of the recipient organism or parental organisms, including information on taxonomic status, common name, origin, centres of origin and centres of genetic diversity, if known, and a description of the habitat where the organisms may persist or proliferate;

(b) Donor organism or organisms. Taxonomic status and common name, source, and the relevant biological characteristics of the donor organisms;

(c) Vector. Characteristics of the vector, including its identity, if any, and its source or origin, and its host range;

(d) Insert or inserts and/or characteristics of modification. Genetic characteristics of the inserted nucleic acid and the function it specifies, and/or characteristics of the modification introduced;

(e) Living modified organism. Identity of the living modified organism, and the differences between the biological characteristics of the living modified organism and those of the recipient organism or parental organisms;

(f) Detection and identification of the living modified organism. Suggested detection and identification methods and their specificity, sensitivity and reliability;

(g) Information relating to the intended use. Information relating to the intended use of the living modified organism, including new or changed use compared to the recipient organism or parental organisms; and

(h) Receiving environment. Information on the location, geographical, climatic and ecological characteristics, including relevant information on biological diversity and centres of origin of the likely potential receiving environment.

Part 5

Annex IIIB of the Proposed European Directive: Information Required In Notifications Concerning Releases of Genetically Modified Higher Plants (GMHPs) (Gymnospermae and Angiospermae)[5]

A) General Information
   1) Name and address of the notifier (company or institute)
   2) Name, qualifications and experience of the responsible scientist(s)
   3) Title of the project

B) Information relating to (A) the recipient or (B) (where appropriate) parental plants
   1) Complete name:
      a) family name;
      b) genus;
      c) species;
      d) subspecies;
      e) cultivar/breeding line;
      f) common name.

   2) (a) Information concerning reproduction:
         (i) mode(s) of reproduction;
         (ii) specific factors affecting reproduction, if any;
         (iii) generation time
      b) Sexual compatibility with other cultivated or wild plant species, including the distribution in Europe of the compatible species.
   3) Survivability:
      a) ability to form structures for survival or dormancy;
      b) specific factors affecting survivability, if any.
   4) Dissemination:
      a) ways and extent (e.g. an estimation of how viable pollen and/or seeds declines with distance) of dissemination;
      b) specific factors affecting dissemination, if any.
   5) Geographical distribution of the plant.
   6) In the case of plant species not normally grown in the Member State(s), description of the natural habitat of the plant, including information on natural predators, parasites, competitors and symbionts.
   7) Other potential interactions,relevant to the GMO, of the plant with organisms in the ecosystem where it is usually grown, or elsewhere, including information on toxic effects on humans, animals and other organisms.

C) Information relating to the genetic modification
   1) Description of the methods used for the genetic modification
   2) Nature and source of the vector used.
   3) Size, source (name) of donor organism(s) and intended function of each

[5] Note: Articles cited in this Part refer to the Proposed Directive and not this report.

D) Information relating to the genetically modified plant

1) Description of the trait(s) and characteristics which have been introduced or modified.
2) Information on the sequences actually inserted/deleted:
   a) size and structure of the insert and methods used for its characterization, including information on any parts of the vector introduced in the GMHP or any carrier or foreign DNA remaining in the GMHP;
   b) in case of deletion, size and function of the deleted region(s);
   c) copy number of the insert;
   d) location(s) of the insert(s) in the plant cells (integrated in the chromosome, chloroplasts, mitochondria, or maintained in a non-integrated form), and methods for its determination;
3) Information on expression of the insert:
   a) information on the developmental expression of the insert during the lifecycle of the plant and methods used for its characterization;
   b) parts of the plant where the insert is expressed (e.g. roots, stem, pollen, etc.).
4) Information on how the genetically modified plant differs from the recipient plant in:
   a) mode(s) and/or rate of reproduction;
   b) dissemination;
   c) survivability.
5) Genetic stability of the insert and phenotypic stability of the GMHP.
6) Any change to the ability of the GMHP to transfer genetic material to other organisms.
7) Information on any toxic, allergenic or other harmful effects on human health arising from the genetic modification.
8) Information on the safety of the GMHP to animal health, particularly regarding any toxic, allergenic or other harmful effects arising from the genetic modification, where the GMHP is intended to be used in animal feedstuffs.
9) Mechanism of interaction between the genetically modified plant and target organisms (if applicable).
10) Potential changes in the interaction of the GMHP with nontarget organisms resulting from the genetic modification.
11) Potential interaction with the abiotic environment.
12) Description of detection and identification techniques for the genetically modified plant.
13) Information about previous releases of the genetically modified plant, if applicable.

E) Information relating to the site of release (only for notifications submitted pursuant to articles 6 and 7)[6]

F) Information relating to the release (only for notifications submitted pursuant to articles 6 and 7)

[6] Article 6 applies to deliberate release of GMOs for any other purpose than for placing on the market, and Article 7 applies to differentiated procedures for GMOs for which sufficient experience has been gained. Neither article is relevant to the present report.

Part 6

Requirements for a Petition for Determination of Non-regulated Status from USDA/APHIS

The USDA/APHIS regulations require that "[t]he petitioner shall include copies of scientific literature, copies of unpublished studies, when available, and data from tests performed upon which to base a determination. The petition shall include all information set forth in paragraph (c) of 7 CFR 340.6...", and that "[a] person shall also include information known to the petitioner which would be unfavorable to a petition."

(c) Required data and information.

The petition shall include the following information:

(1) Description of the biology of the nonmodified recipient plant and information necessary to identify the recipient plant in the narrowest taxonomic grouping applicable.

(2) Relevant experimental data and publications.

(3) A detailed description of the differences in genotype between the regulated article and the nonmodified recipient organism. Include all scientific, common, or trade names, and all designations necessary to identify: the donor organism(s), the nature of the transformation system (vector or vector agent(s)), the inserted genetic material and its product(s), and the regulated article. Include country and locality where the donor, the recipient, and the vector organisms and the regulated articles are collected, developed, and produced.

(4) A detailed description of the phenotype of the regulated article. Describe known and potential differences from the unmodified recipient organism that would substantiate that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived, including but not limited to: Plant pest risk characteristics, disease and pest susceptibilities, expression of the gene product, new enzymes, or changes to plant metabolism, weediness of the regulated article, impact on the weediness of any other plant with which it can interbreed, agricultural or cultivation practices, effects of the regulated article on nontarget organisms, indirect plant pest effects on other agricultural products, transfer of genetic information to organisms with which it cannot interbreed, and any other information which the Administrator believes to be relevant to a determination. Any information known to the petitioner that indicates that a regulated article may pose a greater plant pest risk than the unmodified recipient organism shall also be included.

(5) Field test reports for all trials conducted under permit or notification procedures, involving the regulated article, that were submitted prior to submission of a petition for determination of nonregulated status or prior to submission of a request for extension of a determination of nonregulated status under paragraph (e) of this part. Field test reports shall include the APHIS reference number, methods of observation, resulting data, and

analysis regarding all deleterious effects on plants, nontarget organisms, or the environment.

Part 7

Information Required by the Canadian Food Inspection Agency, Plant Biosafety Office, for Determining Environmental Safety Prior to Approval of Unconfined Release

Applicants should clearly describe the methods used to obtain the requested information together with bibliographic references, including numbered patents, where these are appropriate.

The following information is required:

C1.0 Personnel involved and Status of the PNT [4] in the Application

C1.1 Applicant:

1. Name
2. Address
3. Telephone Number
4. Facsimile Number

C1.2 Canadian representative, if different from above:

1. Name
2. Address
3. Telephone Number
4. Facsimile Number

C1.3 Is the plant material imported? If yes, was an import permit applied for under the Plant Protection Act? Was it granted? If yes, provide the permit number if known. (For information on import permit requirements for PNTs, see Dir96-13.)

C1.4 Was the plant material previously tested in Canada? If yes, in what years?

C1.5 If the PNT was derived through recombinant DNA techniques, were the gene constructs previously tested in Canada? If yes, in what plant species and in what years?

C1.6 Were other government agencies, either foreign or within Canada, notified of the development of the PNT or its important? What was the purpose of such notification?

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4 Plant With Novel Trait. Canada defines this as "a plant variety/genotype possessing characteristics that demonstrate neither familiarity nor substantial equivalence to those present in a distinct, stable population of a cultivated species of seed in Canada and that have been intentionally selected, created or introduced into a population of that species through a specific genetic change. PNT's include those derived from both recombinant DNA technology and plants derived through traditional plant breeding".

C.2.0 Description of the PNT and its Modification

Use Appendix II, Table 1 to summarize this information, where appropriate.

Applicants who have previously applied for, and received, authorization to field test PNT's using the latest version of the Directive Guidelines for the Environmental Release of Plants With Novel Traits Within Confined Field Trials in Canada (Dir2000-07) may refer back to information supplied in Appendix 5, sections 4 "Description of the Plant with Novel (PNT)" and section 5 "Characteristics of the Novel Trait(s)", of that Directive. Please ensure that any questions not previously addressed, are answered in this safety assessment document.

C2.1 Describe the following about the PNT:

1. Confirmation of taxonomy;

2. Designation given to the PNT, including all synonyms;

3. Pedigree information of the PNT (including any relationship to a previously assessed PNT);

4. Give details of the use of the PNT (e.g., to be grown as a field crop for grain production; to be grown as field crop for grain production on lands contaminated with persistent herbicide; to reclaim lands contaminated with heavy metals).

C2.2 Describe the following about the modification:

1. Novel gene products conferring the novel traits

2. Methods used to introduce the novel traits (briefly describe the techniques, if not through recombinant DNA)

3. If the modification was achieved through recombinant DNA techniques:

   a) supply a map of each genetic construct.

   b) for each genetic construct, list, identify source and describe in detail:

      i. genes, including antibiotic resistance, other marker genes or regulatory genes;

      ii. the products of the introduced genes;

      iii. regulatory sequences, i.e., promoters, modifiers, enhancers, signal peptides, and terminators;

      iv. any other DNA sequences.

   c) was the transformation vectorless? If yes, describe in detail.

d) if a vector was employed, answer:

i. what is the vector name and cloning method?

ii. is the vector naturally pathogenic?

iii. was the vector disarmed?

iv. how was the vector disarmed?

v. is there expression of the gene in the vector?

C2.3 In the case of an allopolyploid PNT, in which parental genome is the genetic modification?

C2.4 Number of generations removed from the original modification.

C2.5 Once inserted into the plant, has each genetic modification and its expression been shown to be stable? Provide data demonstrating stability.

C.3.0 Description of the Novel Traits

This information should be summarized in Appendix II, Table 2 and will identify areas of potential interactions of the PNT with the environment. Differences in these interactions from the original unmodified plant or counterpart will be compared in Part D.

C3.1 Characterize in detail the gene products, breakdown products, by-products and their metabolic pathways.

C3.2 Are the gene products tissue-specific?

C3.3 Are the genes expressed during a specific developmental stage?

C3.4 Is gene expression induced? If yes, what are the inducing agents?

C3.5 Describe the activity of the gene products, breakdown products and by-products in the host plant. Describe any changes to existing metabolic pathways (including altered accumulation and storage patterns), including those that might not be intended.

C3.6 The toxicity of the novel gene products, breakdown products and by-products in the environment must be established. Describe:

1. potential toxigenicity to known or potential predators, grazers, parasites, pathogens, competitors and symbiont;

2. potential for adverse human health effects, e.g., exposure to toxins, irritants and antigens. Include estimated level and most likely route of human exposure to the gene products, breakdown products and by-products.

Part D. Biology and Interactions of the PNT

This information is intended to permit a determination and identification of anticipated or observed differences between the PNT and the unmodified form or a named counterpart (closely related genotype, or with a range of named counterparts of similar plant type. See B5.0). Specifically, this is to determine whether there are significantly different/altered interactions with other life forms, resulting from the PNT's novel gene products, which could potentially cause the PNT to become a weed of agriculture, become invasive of natural habitats or be otherwise harmful to the environment.

D1.0 Interactions of the PNT

Information in this section should be used to complete Appendix II, Tables 3 and 4 (again, as required in Part C, applicants should clearly describe the methods used to obtain the information together with bibliographic references, including numbered patents, where these are appropriate. In addition, data should be obtained from experimental designs using sound statistical methods, where appropriate). In completing the tables, applicants may consider it unnecessary or inappropriate to provide certain information. In these instances, information requirements may be waived if valid scientific rationale is provided.

D1.1 Relative phenotype expression of the PNT (species replacement or competition studies may be appropriate when there is reason to believe that the biology of the plant has been altered in unpredictable ways). See Appendix III:

1. Reproductive and survival biology;

2. Adaptations to stress factors (for biotic stress factors, identify those life forms with which the PNT interacts differently);

3. Biochemistry: For novel gene products identified in Part C3.6 that are known to be toxic, describe:

   a) likelihood and change of level of exposure of consumers and symbiont;

   b) the effect on soil micro flora and fauna. Residual studies may be conducted to determine macro changes. Observed changes at this level may warrant further in-depth studies (See Appendix IV)

D1.2 Agricultural-Silvicultural Practices:

1. What are the proposed release sites for the PNT?

   a) all of Canada?

   b) specific regions?

   c) what is the projected area (ha) of release?

2. Will the modification result in the PNT being grown outside of the normal geographic production area for the species?

3. Will the modification result in the PNT being grown outside of the usual habitat (e.g., cultivated agricultural lands) for the species?

4. Will the cultivation practices (land preparation, weed and pest control, harvest, and post-harvest protocols) involved in growing the PNT vary from those traditionally used?

   (a) if no: no further information required

   (b) if yes:

      (i) describe the change in cultivation practices;

      (ii) provide information showing the effect of these changes on sustainability, especially with respect to pesticide use, frequency of tillage, soil erosion and consequential changes in energy and soil conservation;

   (c) will volunteer plants of the PNT result in altered cultivation practices for succeeding crops?

5. If it is anticipated that the PNT will be cultivated only under contract/controlled conditions, describe:

   (a) any control and mitigation procedures;

   (b) post-harvest procedures, including procedures for disposal of remaining plant matter.

6. What deployment strategies are to be used? (for example, in forestry, the avoidance of monocultures).

D1.3 Potential environmental effects resulting from introgression.

Where there is potential for gene flow from the PNT into related species, detail the consequences of novel gene introgression into those species and resulting expression. Interactions identified for the original PNT should be considered, as appropriate, for these species.

Part 8

Consistency of the Information Requirements of Selected Regions or Countries with the Risk Assessment Points to Consider Provided in Annex III of the Cartagena Biosafety Protocol of the Convention on Biodiversity.

(a) Recipient organism or parental organisms. The biological characteristics of the recipient organism or parental organisms, including information on taxonomic status, common name, origin, centres of origin and centres of genetic diversity, if known, and a description of the habitat where the organisms may persist or proliferate;

European Directive

B. Information relating to (A) the recipient or (B) (where appropriate) parental plants

1. Complete name:
   (a) family name;
   (b) genus;
   (c) species;
   (d) subspecies;
   (e) cultivar/breeding line;
   (f) common name.
2. (a) Information concerning reproduction:
   i. mode(s) of reproduction;
   ii. specific factors affecting reproduction, if any;
   iii. generation time
   (b) Sexual compatibility with other cultivated or wild plant species, including the distribution in Europe of the compatible species.
3. Survivability:
   (a) ability to form structures for survival or dormancy;
   (b) specific factors affecting survivability, if any.
4. Dissemination:
   (a) ways and extent (e.g. an estimation of how viable pollen and/or seeds declines with distance) of dissemination;
   (b) specific factors affecting dissemination, if any.
5. Geographical distribution of the plant.
6. In the case of plant species not normally grown in the Member State(s), description of the natural habitat of the plant, including information on natural predators, parasites, competitors and symbionts.
7. Other potential interactions,relevant to the GMO, of the plant with organisms in the ecosystem where it is usually grown, or elsewhere, including information on toxic effects on humans, animals and other organisms.

United States (USDA)

Description of the biology of the nonmodified recipient plant and information necessary to identify the recipient plant in the narrowest taxonomic grouping applicable.

Canada (CFIA)

Not specifically required; this information will be inherent in the descriptions of the plant with novel traits.

(b) <u>Donor organism or organisms</u>. Taxonomic status and common name, source, and the relevant biological characteristics of the donor organisms;

European Directive

C. Information relating to the genetic modification
   3. Size, source (name) of donor organism(s) and intended function of each constituent fragment of the region intended for insertion.

United States (USDA)

A detailed description of the differences in genotype between the regulated article and the nonmodified recipient organism. Include all scientific, common, or trade names, and all designations necessary to identify: the donor organism(s), the nature of the transformation system (vector or vector agent(s)), the inserted genetic material and its product(s), and the regulated article. Include country and locality where the donor, the recipient, and the vector organisms and the regulated articles are collected, developed, and produced.

Canada (CFIA)

C2.2 Describe the following about the modification:

1. Novel gene products conferring the novel traits

2. Methods used to introduce the novel traits (briefly describe the techniques, if not through recombinant DNA)

3. If the modification was achieved through recombinant DNA techniques:

   (a) supply a map of each genetic construct.

   (b) for each genetic construct, list, identify source and describe in detail:

      i. genes, including antibiotic resistance, other marker genes or regulatory genes;

      ii. the products of the introduced genes;

      iii. regulatory sequences, i.e., promoters, modifiers, enhancers, signal peptides, and terminators;

      iv. any other DNA sequences.

   (c) was the transformation vectorless? If yes, describe in detail.

   (d) if a vector was employed, answer:

      i. what is the vector name and cloning method?

ii. is the vector naturally pathogenic?

iii. was the vector disarmed?

iv. how was the vector disarmed?

v. is there expression of the gene in the vector?

(c) Vector. Characteristics of the vector, including its identity, if any, and its source or origin, and its host range;

European Directive

C. Information relating to the genetic modification
1. Description of the methods used for the genetic modification
2. Nature and source of the vector used.
3. Size, source (name) of donor organism(s) and intended function of each constituent fragment of the region intended for insertion.

United States (USDA)

A detailed description of the differences in genotype between the regulated article and the nonmodified recipient organism. Include all scientific, common, or trade names, and all designations necessary to identify: the donor organism(s), the nature of the transformation system (vector or vector agent(s)), the inserted genetic material and its product(s), and the regulated article. Include country and locality where the donor, the recipient, and the vector organisms and the regulated articles are collected, developed, and produced.

Canada (CFIA)

C2.2 Describe the following about the modification:

3. If the modification was achieved through recombinant DNA techniques:

(a) supply a map of each genetic construct.

(b) for each genetic construct, list, identify source and describe in detail:

i. genes, including antibiotic resistance, other marker genes or regulatory genes;

ii. the products of the introduced genes;

iii. regulatory sequences, i.e., promoters, modifiers, enhancers, signal peptides, and terminators;

iv. any other DNA sequences.

(c) was the transformation vectorless? If yes, describe in detail.

(d) if a vector was employed, answer:

i. what is the vector name and cloning method?

ii. is the vector naturally pathogenic?

iii. was the vector disarmed?

iv. how was the vector disarmed?

v. is there expression of the gene in the vector?

(d) <u>Insert or inserts and/or characteristics of modification</u>. Genetic characteristics of the inserted nucleic acid and the function it specifies, and/or characteristics of the modification introduced;

European Directive

D. Information relating to the genetically modified plant
   1. Description of the trait(s) and characteristics which have been introduced or modified.
   2. Information on the sequences actually inserted/deleted:
      (a) size and structure of the insert and methods used for its characterization, including information on any parts of the vector introduced in the GMHP or any carrier or foreign DNA remaining in the GMHP;
      (b) in case of deletion, size and function of the deleted region(s);
      (c) copy number of the insert;
      (d) location(s) of the insert(s) in the plant cells (integrated in the chromosome, chloroplasts, mitochondria, or maintained in a non-integrated form), and methods for its determination;
   3. Information on expression of the insert:
      (a) information on the developmental expression of the insert during the lifecycle of the plant and methods used for its characterization;
      (b) parts of the plant where the insert is expressed (e.g. roots, stem, pollen, etc.).

United States (USDA)

A detailed description of the differences in genotype between the regulated article and the nonmodified recipient organism. Include all scientific, common, or trade names, and all designations necessary to identify: the donor organism(s), the nature of the transformation system (vector or vector agent(s)), the inserted genetic material and its product(s), and the regulated article. Include country and locality where the donor, the recipient, and the vector organisms and the regulated articles are collected, developed, and produced.

Canada (CFIA)

C2.5 Once inserted into the plant, has each genetic modification and its expression been shown to be stable? Provide data demonstrating stability.

(e) <u>Living modified organism</u>. Identity of the living modified organism, and the differences between the biological characteristics of the living modified organism and those of the recipient organism or parental organisms;

European Directive

D. Information relating to the genetically modified plant

4. Information on how the genetically modified plant differs from the recipient plant in:
   (a) mode(s) and/or rate of reproduction;
   (b) dissemination;
   (c) survivability.
5. Genetic stability of the insert and phenotypic stability of the GMHP.
6. Any change to the ability of the GMHP to transfer genetic material to other organisms.
7. Information on any toxic, allergenic or other harmful effects on human health arising from the genetic modification.
8. Information on the safety of the GMHP to animal health, particularly regarding any toxic, allergenic or other harmful effects arising from the genetic modification, where the GMHP is intended to be used in animal feedstuffs.
9. Mechanism of interaction between the genetically modified plant and target organisms (if applicable).
10. Potential changes in the interaction of the GMHP with nontarget organisms resulting from the genetic modification.
11. Potential interaction with the abiotic environment.
12. Description of detection and identification techniques for the genetically modified plant.
13. Information about previous releases of the genetically modified plant, if applicable.

United States (USDA)

A detailed description of the differences in genotype between the regulated article and the nonmodified recipient organism. Include all scientific, common, or trade names, and all designations necessary to identify: the donor organism(s), the nature of the transformation system (vector or vector agent(s)), the inserted genetic material and its product(s), and the regulated article. Include country and locality where the donor, the recipient, and the vector organisms and the regulated articles are collected, developed, and produced.

A detailed description of the phenotype of the regulated article. Describe known and potential differences from the unmodified recipient organism that would substantiate that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived, including but not limited to: Plant pest risk characteristics, disease and pest susceptibilities, expression of the gene product, new enzymes, or changes to plant metabolism, weediness of the regulated article, impact on the weediness of any other plant with which it can interbreed, agricultural or cultivation practices, effects of the regulated article on nontarget organisms, indirect plant pest effects on other agricultural products, transfer of genetic information to organisms with which it cannot interbreed, and any other information which the Administrator believes to be relevant to a determination. Any information known to the petitioner that indicates that a regulated article may pose a greater plant pest risk than the unmodified recipient organism shall also be included.

Canada (CFIA)

C.2.0 Description of the PNT and its Modification

C2.1 Describe the following about the PNT:

1. Confirmation of taxonomy;
2. Designation given to the PNT, including all synonyms;
3. Pedigree information of the PNT (including any relationship to a previously assessed PNT);
4. Give details of the use of the PNT (e.g., to be grown as a field crop for grain production; to be grown as field crop for grain production on lands contaminated with persistent herbicide; to reclaim lands contaminated with heavy metals).

C2.2 Describe the following about the modification:

1. Novel gene products conferring the novel traits
2. Methods used to introduce the novel traits (briefly describe the techniques, if not through recombinant DNA)
3. If the modification was achieved through recombinant DNA techniques:
   - (a) supply a map of each genetic construct.
   - (b) for each genetic construct, list, identify source and describe in detail:
     - i. genes, including antibiotic resistance, other marker genes or regulatory genes;
     - ii. the products of the introduced genes;
     - iii. regulatory sequences, i.e., promoters, modifiers, enhancers, signal peptides, and terminators;
     - iv. any other DNA sequences.
   - (c) was the transformation vectorless? If yes, describe in detail.
   - (d) if a vector was employed, answer:
     - i. what is the vector name and cloning method?
     - ii. is the vector naturally pathogenic?
     - iii. was the vector disarmed?
     - iv. how was the vector disarmed?
     - v. is there expression of the gene in the vector?

C2.3 In the case of an allopolyploid PNT, in which parental genome is the genetic modification?

C2.4 Number of generations removed from the original modification.

C2.5 Once inserted into the plant, has each genetic modification and its expression been shown to be stable? Provide data demonstrating stability.

C.3.0 Description of the Novel Traits

C3.1 Characterize in detail the gene products, breakdown products, by-products and their metabolic pathways.

C3.2 Are the gene products tissue-specific?

C3.3 Are the genes expressed during a specific developmental stage?

C3.4 Is gene expression induced? If yes, what are the inducing agents?

C3.5 Describe the activity of the gene products, breakdown products and by-products in the host plant. Describe any changes to existing metabolic pathways (including altered accumulation and storage patterns), including those that might not be intended.

C3.6 The toxicity of the novel gene products, breakdown products and by-products in the environment must be established. Describe:

1. potential toxigenicity to known or potential predators, grazers, parasites, pathogens, competitors and symbiont;
2. potential for adverse human health effects, e.g., exposure to toxins, irritants and antigens. Include estimated level and most likely route of human exposure to the gene products, breakdown products and by-products.

D1.0 Interactions of the PNT

D1.1 Relative phenotype expression of the PNT (species replacement or competition studies may be appropriate when there is reason to believe that the biology of the plant has been altered in unpredictable ways).

1. Reproductive and survival biology;
2. Adaptations to stress factors (for biotic stress factors, identify those life forms with which the PNT interacts differently);
3. Biochemistry: For novel gene products identified in Part C3.6 that are known to be toxic, describe:
   (a) likelihood and change of level of exposure of consumers and symbiont;
   (b) the effect on soil micro flora and fauna. Residual studies may be conducted to determine macro changes. Observed changes at this level may warrant further in-depth studies (See Appendix IV)

(f) Detection and identification of the living modified organism. Suggested detection and identification methods and their specificity, sensitivity and reliability;

European Directive

D. Information relating to the genetically modified plant
   1. Description of detection and identification techniques for the genetically modified plant.

United States (USDA)

A detailed description of the differences in genotype between the regulated article and the nonmodified recipient organism. Include all scientific, common, or trade names, and all designations necessary to identify: the donor organism(s), the nature of the transformation system (vector or vector agent(s)), the inserted genetic material and its product(s), and the regulated article. Include country and locality where the donor, the recipient, and the vector organisms and the regulated articles are collected, developed, and produced.

Canada (CFIA)

Not specifically required

(g) <u>Information relating to the intended use</u>. Information relating to the intended use of the living modified organism, including new or changed use compared to the recipient organism or parental organisms

European Directive

Not specifically required; this information will be inherent in the descriptions of the genetic modification.

United States (USDA)

A detailed description of the phenotype of the regulated article. Describe known and potential differences from the unmodified recipient organism that would substantiate that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived, including but not limited to: Plant pest risk characteristics, disease and pest susceptibilities, expression of the gene product, new enzymes, or changes to plant metabolism, weediness of the regulated article, impact on the weediness of any other plant with which it can interbreed, agricultural or cultivation practices, effects of the regulated article on nontarget organisms, indirect plant pest effects on other agricultural products, transfer of genetic information to organisms with which it cannot interbreed, and any other information which the Administrator believes to be relevant to a determination. Any information known to the petitioner that indicates that a regulated article may pose a greater plant pest risk than the unmodified recipient organism shall also be included.

Canada (CFIA)

D1.2 Agricultural-Silvicultural Practices:

1. What are the proposed release sites for the PNT?
   (a) all of Canada?
   (b) specific regions?
   (c) what is the projected area (ha) of release?
2. Will the modification result in the PNT being grown outside of the normal geographic production area for the species?
3. Will the modification result in the PNT being grown outside of the usual habitat (e.g., cultivated agricultural lands) for the species?
4. Will the cultivation practices (land preparation, weed and pest control, harvest, and post-harvest protocols) involved in growing the PNT vary from those traditionally used?
   (a) if no: no further information required
   (b) if yes:
      i. describe the change in cultivation practices;
      ii. provide information showing the effect of these changes on sustainability, especially with respect to pesticide use, frequency of tillage, soil erosion and consequential changes in energy and soil conservation;
   (c) will volunteer plants of the PNT result in altered cultivation practices for succeeding crops?

5. If it is anticipated that the PNT will be cultivated only under contract/controlled conditions, describe:
   (a) any control and mitigation procedures;
   (b) post-harvest procedures, including procedures for disposal of remaining plant matter.
6. What deployment strategies are to be used? (for example, in forestry, the avoidance of monocultures).

D1.3 Potential environmental effects resulting from introgression.

Where there is potential for gene flow from the PNT into related species, detail the consequences of novel gene introgression into those species and resulting expression. Interactions identified for the original PNT should be considered, as appropriate, for these species.

(h) <u>Receiving environment</u>. Information on the location, geographical, climatic and ecological characteristics, including relevant information on biological diversity and centres of origin of the likely potential receiving environment.

European Directive

Information relating to the genetically modified plant

9. Mechanism of interaction between the genetically modified plant and target organisms (if applicable).
10. Potential changes in the interaction of the GMHP with nontarget organisms resulting from the genetic modification.
11. Potential interaction with the abiotic environment.

United States (USDA)

(Required by U.S. law (National Environmental Protection Act) to be included in the Environmental Assessment conducted by USDA/APHIS. USDA/APHIS relies on information it has, and can request it from the applicant):

A detailed description of the phenotype of the regulated article. Describe known and potential differences from the unmodified recipient organism that would substantiate that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived, including but not limited to: Plant pest risk characteristics, disease and pest susceptibilities, expression of the gene product, new enzymes, or changes to plant metabolism, weediness of the regulated article, impact on the weediness of any other plant with which it can interbreed, agricultural or cultivation practices, effects of the regulated article on nontarget organisms, indirect plant pest effects on other agricultural products, transfer of genetic information to organisms with which it cannot interbreed, and any other information which the Administrator believes to be relevant to a determination. Any information known to the petitioner that indicates that a regulated article may pose a greater plant pest risk than the unmodified recipient organism shall also be included.

Canada (CFIA)

D1.3 Potential environmental effects resulting from introgression.

Where there is potential for gene flow from the PNT into related species, detail the consequences of novel gene introgression into those species and resulting expression. Interactions identified for the original PNT should be considered, as appropriate, for these species.

Part 9

Application of Environmental Safety Guidelines to the Environmental Safety Assessment of Potato Variety "Spunta" Genetically Engineered to be Resistant to Potato Tuber Moth

I. Information on the recipient plant or parental plants.

Information on *Solanum tuberosum* addressing questions in this section can be found in resource documents written by regulatory bodies including the Organization for Economic Cooperation and Development (OECD, 1997), the Canadian Food Inspection Agency (CFIA, 1996), and the US Department of Agriculture (USDA, 2001). These resource documents are attached as Annex VII. The National Biosafety Committee may determine the necessity for additional information, in particular any information that might be lacking on the cultivar itself or on with regard to applicability to Egypt.

A. Taxonomy:

(a) family name: Solanaceae

(b) genus: Solanum

(c) species: tuberosum

(d) subspecies: tuberosum

(e) cultivar/breeding line: Spunta

(f) common name: Potato

B. Reproductive characteristics of the recipient plant

1. Information concerning reproduction:

a. mode(s) of reproduction;

See resource documents

b. specific factors affecting reproduction, if any;

See resource documents

c. generation time

See resource documents

2. Sexual compatibility with other cultivated or wild plant species, including the distribution in Egypt of compatible species.

See resource documents, particularly OECD 1997. No compatible wild species are known in Egypt.

C. Survivability:

1. ability to form structures for survival or dormancy;

   See resource documents

2. specific factors affecting survivability, if any.

   See OECD 1997

D. Allelopathy

Spunta is a cultivated potato variety. It is assumed that no allelopathic effects have been observed for this variety throughout its history of use in Egypt. However, if any information contrary to that assumption does exist, it should be given here.

E. Dormancy

Potatoes normally reproduce by tubers, which can remain viable for long periods of time (OECD, 1997). However, it can reproduce by seeds, which also have long periods of viability (OECD, 1997). Long viability, however, does not necessarily indicate dormancy.

F. Dissemination (pollen, seed, vegetative means):

1. ways and extent (e.g. an estimation of how viable pollen and/or seeds declines with distance) of dissemination;

   See resource documents.

2. specific factors affecting dissemination, if any.

   See resource documents.

E. Geographical distribution of the plant, including the centers of origin and genetic diversity, if known.

See resource documents

F. In the case of plant species not normally grown in Egypt, description of the natural habitat of the plant, including information on natural predators, parasites, competitors and symbionts.

Potato is normally grown in Egypt.

G. Information on the location, geographical, climatic and ecological characteristics, including relevant information on biological diversity and centers of origin of the likely potential receiving environment

Documents containing information describing general ecological characteristics of Egypt have been written (see for example MOE, 1997). Other published sources of information as well as specific reports, such as the inventories of plants, animals, and

micro-organisms mentioned in the MOE 1997 report would be useful for fulfilling this data requirement.

H. Other potential interactions of the plant with organisms in the ecosystem where it is usually grown, or elsewhere, including information on toxic effects on humans, animals and other organisms.

Steroidal glycoalkaloids are known to exist in potato, and have an effect on humans and animals.

II. The sources of the new genetic materials (donor organisms and vectors), their characterization in the genetically engineered plant.

Much of the information in this section was already described in a previous report (Quemada et al., 2001). Therefore, that report will be referenced here.

A. Information on the donor organism(s) and, when appropriate, on other members closely related to the donor organism(s), including:

1. its scientific name
   Provided in Annex III of Quemada et al. (2001)
2. taxonomic classification
   Provided in Annex III of Quemada et al. (2001)
3. usual or common name.
   Provided in Annex III of Quemada et al. (2001)

B. Information on all genetic material potentially delivered to the engineered plant:

1. The specific method used for transformation.
   Provided in Annex III of Quemada et al. (2001)
2. The vector DNA, including the source (e.g. plant, microbial, viral , synthetic), identity and expected function in the plant.
   A detailed map of the vector plasmid would be provided. In the case of Spunta G2 and Spunta G3, the map of pSPUD5 would be given along with an indication of the origin of the various elelments in the map.
3. Intermediate host organisms including the organisms (e.g. bacteria) used to produce or process DNA for transformation of the host organism
   Since engineering of pSPUD5 was probably conducted in *E. coli*, the *E. coli* host strains should be reported.
4. The DNA to be introduced (other than the vector), including:
   a. the characterization of all the genetic components including marker genes, regulatory and other elements affecting the function of the DNA
   b. the size and identity
   c. the location and orientation of the sequence in the final vector/construct
   d. the function

   This information would be included in the detailed map provided in II.B.2. above. It the actual DNA to be inserted differs from the entire vector, the intended fragment would be indicated.

C. Molecular and biochemical characterization of the DNA actually inserted into the plant genome, including:

1. The number of insertion sites and location (e.g. chromosomal, chloroplast, mitochondrial

2. The organisation of the inserted genetic material at each insertion site, including sequence data of the inserted material and of the surrounding region if deemed necessary. Information should be presented regarding the arrangement of the genetic material used for transformation has been conserved or whether significant rearrangements have occurred upon integration.

   Information for 1 and 2 would be provided by a thorough Southern blot analysis of Spunta G2 and Spunta G3. The National Biosafety Committee would decide whether sequencing of the inserts would be necessary to resolve any safety concerns.

3. The level and site of expression in the plant of the introduced gene product(s),

   Expression data as required for food and feed safety, and with guidance from documents such as those in Annex VIII, would be provided .

4. Data to demonstrate that all introduced traits are stably expressed and inherited.

   For potato, data would be provided to demonstrate that the transgene trait is stably maintained and expressed during vegetative propagation over a number of cycles that is appropriate to the crop.

III. <u>Information on the genetically engineered plant and its effect on the receiving environment</u>.

Information relating to known and potential differences between the genetically modified plant and the recipient plant in:

A. Plant pest risk characteristics

Spunta G2 and Spunta G3 are not engineered to express any new characteristics that would raise concerns about their being a plant pest risk. For example, they are not engineered to produce new compounds that would be toxic to animals or humans, nor have they been engineered with characteristics intended to make them out-compete other plant species.

B. Disease and pest susceptibilities

Common pests of potato are known (see OECD, 1997). Spunta G2 and Spunta G3 should be assessed for any difference in susceptibility to pests normally occurring in Egypt. In addition, differences in susceptibility to diseases of potato in Egypt (e.g. brown rot) should be assessed. The names of the diseases and the scientific names of the pathogens should be provided for each disease tested. Disease and pathogen susceptibility should preferably be obtained from natural infestations, but care should be taken to obtain results that have statistical validity.

C. Expression of the gene product

The gene product is a protein that is not expressed in non-genetically engineered Spunta. Therefore, the expression of this gene in any tissues of Spunta G2 and Spunta G3 is clearly different from non-genetically engineered Spunta.

D. New enzymes

The marker gene, *npt2*, encodes neomycin phosphotransferase 2, an enzyme new to potato. Any other new enzymatic activity detected, or any altered activity of any enzymes that are important for quality in potato (e.g. starch metabolizing enzymes) would be assessed. These studies would normally be conducted in order to characterize thoroughly the potato, with respect to its suitability for food use as well as for any industrial uses in Egypt (e.g. starch production).

E. Changes to plant metabolism

Any observed differences in plant metabolism, for example, nutrient requirements, differential sensitivity or resistance to herbicides, or new secondary compounds should be reported. In particular, the information on steroid glycoalkaloids generated for the food safety assessment should also be considered. The National Biosafety Committee should determine if specific metabolic aspects should be investigated.

F. Weediness of the genetically engineered plant, including
1. information concerning reproduction:
   a. mode(s) of reproduction
   b. specific factors affecting reproduction, if any
   c. generation time
2. dissemination (pollen, seed, vegetative means)
   a. ways and extent (e.g. an estimation of how viable pollen seeds declines with distance) of dissemination
   b. specific factor affecting dissemination, if any
3. survivability.
   a. ability to form structures for survival or dormancy
   b. specific factors affecting survivability, if any
4. allelopathy
5. dormancy

Spunta G2 and Spunta G3 should be compared with non-genetically engineered Spunta with respect to the characteristics above, and any differences noted. Quantitative data should be gathered using experimental designs that are statistically sound. Information may also be obtained through relevant field surveys or through the published literature.

F. Impact on the weediness of any other plant with which it can interbreed

This requirement is not applicable to potato, since it cannot interbreed with any other plant in Egypt.

H. Intended use and agricultural or cultivation practices, including where applicable,
1. altered geographic range of cultivation

2. altered habitat adaptation
3. altered cultivation practices, including
    a. information showing the effect of these changes on sustainability, especially with respect to pesticide use, frequency of tillage, soil erosion and consequential changes in energy and soil conservation
    b. information on any effect of volunteer plants on crop rotation practices
    c. specific containment or postharvest procedures, including procedures for disposal of remaining plant matter.
        i. any specified conditions for isolation as standard operating procedure for production
        ii. any specified conditions for handling crop rotations as standard operating procedure for production
        iii. any specified conditions for dealing with volunteers as standard operating procedure for production
    d. planting practices and policies (e.g. planting of mixtures to delay the evolution of resistance).

Any differences in the cultivation of Spunta G2 and Spunta G3 compared to non-genetically engineered Spunta should be provided. For example if the new trait will result in the cultivation of Spunta G2 and Spunta G3 in regions where non-genetically engineered Spunta is currently not being grown due to potato tuber moth infestation, that information should be provided. Similarly, if Spunta G2 and Spunta G3 display any ability to grow in new habitats, such as more arid or more saline areas, those characteristics should be noted. Furthermore, if there are proposed planting practices and policies to deal with the evolution of resistance to cry5, those policies and practices should be provided. Any effect on the use of pesticides should be noted, preferably with data to demonstrate any claims for reduced pesticide usage.

Replicated observations would be needed. If the characteristics of Spunta G2 and Spunta G3 require the grower to plant, harvest, or employ post-harvest practices that are different from non-genetically engineered Spunta, that information should be provided. In particular, if there are proposed conditions of planting in order to manage the evolution of resistance to the *cry5* gene product, that information should be provided here.

I. Effects of the genetically engineered plant on non-target organisms. If the introduced gene product is known to act by a toxic mechanism, the effects on potential non-target organisms that might be affected by such a mechanism should be evaluated, including soil micro flora and fauna.

The effect of Spunta G2 and Spunta G3 on non-target organisms can be assessed (among other ways) by conducting comparisons between species found on these transgenic lines and non-genetically engineered Spunta. Similarly, a census of soil micro flora and fauna would be conducted to determine any changes. Replicated observations would be needed.

J. Indirect plant pest effects on other agricultural products

If any difference between Spunta G2, Spunta G3 and non-genetically engineered Spunta is observed with respect to their ability to be a pest in other crops, for example in their ability to volunteer (thus interfering with crop rotations), that information should be provided.

K. Transfer of genetic information to organisms with which it cannot interbreed

This requirement asks the applicant to provide any information that would raise concerns that horizontal gene transfer would be any different between Spunta G2 and Spunta G3 and non-genetically engineered Spunta. Typically, if certain sequences that would enhance DNA mobility into microorganisms are absent in the introduced DNA, then no testing should be necessary. However, if those sequences are present, the National Biosafety Committee might require that tests be done. The specific test protocols should be determined in consultation with the committee.

L. Potential interaction with the abiotic environment.

Any information that would be relevant to any difference between Spunta G2 and Spunta G3 and non-transgenic Spunta with respect to their interaction with the soil or other abiotic components of the environment would be provided here. This question is a difficult one to answer; typically if the characteristics of the genetically engineered and non-genetically engineered plants are substantially equivalent, no differences in their interaction with the abiotic environment would be expected.

M. Description of detection and identification techniques for the genetically modified plant.

An indication of techniques that would be appropriate for monitoring the presence of the genetically engineered plant in the environment would be provided here in order to assist with any post-approval monitoring work that might be required.

N. Information about previous releases of the genetically modified plant, if applicable, including past reports provided to other regulatory agencies

If reports and publications have been written about Spunta G3 and Spunta G3, specifically reports that concern field trials both in Egypt and other countries, that information should be included.

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Annex 1

Resource Documents on the Biology of Potato from OECD, CFIA, and USDA

Series on Harmonization of Regulatory Oversight in Biotechnology No. 8

CONSENSUS DOCUMENT ON THE BIOLOGY OF SOLANUM TUBEROSUM SUBSP. TUBEROSUM (POTATO)

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

Paris

56030

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No. 3, *Report of the OECD Workshop on the Commercialisation of Agricultural Products Derived through Modern Biotechnology* (1995)

No. 4, *Industrial Products of Modern Biotechnology Intended for Release to the Environment: The Proceedings of the Fribourg Workshop* (1996)

No. 5, *Consensus Document on General Information concerning the Biosafety of Crop Plants Made Virus Resistant through Coat Protein Gene-Mediated Protection* (1996)

No. 6, *Consensus Document on Information Used in the Assessment of Environmental Applications Involving* Pseudomonas (1997)

No. 7, *Consensus Document on the Biology of* Brassica Napus L. *(Oilseed Rape)* (1997)

Consensus Document on Information Used in the Assessment of Environmental Applications Involving Rhizobiacea (in preparation)

Consensus Document on Information Used in the Assessment of Environmental Applications Involving Bacillus (in preparation)

Consensus Document on the Biology of Triticum aestivum *(wheat)* (in preparation)

Consensus Document on the Biology of Oryza sativa *(rice)* (in preparation)

Consensus Document on the Biology of Picea abies L. *(Norway Spruce)* (in preparation)

Consensus Document on the Biology of Picea glauca (Moench) Voss *(White Spruce)* (in preparation)

Consensus Document on the Biology of Populus L. *(poplars)* (in preparation)

Consensus Document on General Information Concerning the Genes and their Enzymes which Confer Tolerance to Phosphinothricin Herbicides (in preparation)

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43

OECD Environmental Health and Safety Publications

Series on Harmonization of Regulatory Oversight in Biotechnology

No. 8

Consensus Document on the Biology of *Solanum tuberosum* subsp. *tuberosum* (Potato)

Environment Directorate

Organisation for Economic Co-operation and Development

Paris 1997

44

About the OECD

The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 29 industrialised countries in North America, Europe and the Pacific, as well as the European Commission, meet to co-ordinate and harmonize policies, discuss issues of mutual concern, and work together to respond to international problems. Most of the OECD's work is carried out by more than 200 specialized Committees and subsidiary groups composed of Member country delegates. Observers from several countries with special status at the OECD, and from interested international organisations, attend many of the OECD's Workshops and other meetings. Committees and subsidiary groups are served by the OECD Secretariat, located in Paris, France, which is organised into Directorates and Divisions.

The Environmental Health and Safety Division publishes free-of-charge documents in six different series: **Testing and Assessment; Good Laboratory Practice and Compliance Monitoring; Pesticides; Risk Management; Harmonization of Regulatory Oversight in Biotechnology; and Chemical Accidents.** More information about the Environmental Health and Safety Programme and EHS publications is available on the OECD's World Wide Web site (see below).

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45

FOREWORD

The OECD's Expert Group on Harmonization of Regulatory Oversight in Biotechnology decided at its first session, in June 1995, to focus its work on the development of ***consensus documents*** which are mutually acceptable among Member countries. These consensus documents contain information for use during the regulatory assessment of a particular product. In the area of plant biosafety, consensus documents are being initiated on the biology of certain crop plants and on selected traits.

This document, which was prepared by the Netherlands as lead country in collaboration with the United Kingdom, addresses the biology of the crop plant *Solanum tuberosum* subsp. *tuberosum* (potato). It has been revised based on comments received from OECD countries and on subsequent comments from National Co-ordinators following a second round of review in January 1997.

As part of a joint project with the United Nations Industrial Development Organization (UNIDO) on centres of origin of diversity, the document was reviewed by experts in several countries in South America. Relevant comments submitted by these experts have been incorporated.

The Joint Meeting of the Chemicals Group and Management Committee of the Special Programme on the Control of Chemicals has recommended that this document be made available to the public. It is published on the authority of the Secretary-General of the OECD.

TABLE OF CONTENTS

Preamble 9

Section I General information 11

Section II Taxonomic status 12

Section III Centre of diversity 13

Section IV Identification methods 14

A. Morphology and somaclonal variation 14

B. Molecular identification 15

Section V Genetic characteristics: the genome 16

Section VI Reproductive biology 17

A. Sexual reproduction 17

B. Asexual reproduction 18

Section VII Crosses 19

A. Intraspecific crosses 19

B. Interspecific crosses: crosses within the subsection *potatoe* (tuber-bearing potatoes) 19

C. Interspecific crosses: crosses with species from sections other than *petota* 21

Section VIII Ecology of *Solanum tuberosum* subsp. *tuberosum* 22

A. Cultivation 22

B. Volunteers and weediness 23

(continued next page)

Section IX References 24

Annex I Ploidy and EBN of most *Solanum* species within the section *petota* 29

Annex II The most common diseases in *Solanum tuberosum* subsp. *tuberosum* and their distribution 33

Questionnaire to return to the OECD 37

48

PREAMBLE

OECD Member countries are moving rapidly towards the commercialisation and marketing of agricultural and industrial products of modern biotechnology. They have therefore identified the need for harmonization of regulatory approaches to the assessment of these products, in order to avoid unnecessary trade barriers.

In 1993, **Commercialisation of Agricultural Products Derived through Modern Biotechnology** was instituted as a joint project of the OECD's Environment Policy Committee and Committee on Agriculture. The objective of this project is to assist countries in their regulatory oversight of agricultural products derived through modern biotechnology – specifically in their efforts to ensure safety, to make oversight policies more transparent and efficient, and to facilitate trade. The project is focused on the review of national policies, with respect to regulatory oversight, that will affect the movement of these products into the marketplace.

The first step in this project was to carry out a survey concentrating on national policies with regard to regulatory oversight of these products. Data requirements for products produced through modern biotechnology, and mechanisms for data assessment, were also surveyed. The results were published in *Commercialisation of Agricultural Products Derived through Modern Biotechnology: Survey Results* (OECD, 1995).

Subsequently, an OECD Workshop was held in June 1994 in Washington, D.C. with the aims of improving awareness and understanding of the various systems of regulatory oversight developed for agricultural products of biotechnology; identifying similarities and differences in various approaches; and identifying the most appropriate role for the OECD in further work towards harmonization of these approaches. Approximately 80 experts in the areas of environmental biosafety, food safety and varietal seed certification, representing 16 OECD countries, eight non-member countries, the European Commission and several international organisations, participated in the Workshop. *Report of the OECD Workshop on the Commercialisation of Agricultural Products Derived through Modern Biotechnology* was also published by the OECD in 1995.

As a next step towards harmonization, the Expert Group on Harmonization of Regulatory Oversight in Biotechnology instituted the development of ***consensus documents*** which are ***mutually acceptable*** among Member countries. The purpose of these documents is to identify common elements in the safety assessment of a new plant variety developed through modern biotechnology, in order to encourage information sharing and prevent duplication of effort among countries. These common elements fall into two general categories: the biology of the host species, or crop; and the gene product. *Solanum tuberosum* subsp. *tuberosum* (potato) is the second crop plant chosen for review, the first having been *Brassica napus* L. (oilseed rape).[1] As part of a joint project with the United Nations Industrial Development Organization (UNIDO) on centres of origin of diversity, this consensus document was reviewed by experts in several countries in South America. Relevant comments submitted by these experts have been incorporated.

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[1] See *Consensus Document on the Biology of* Brassica napus *L. (Oilseed Rape)*, Series on Harmonization of Regulatory Oversight in Biotechnology No. 7 (OECD, 1997). The consensus documents and many other publications prepared by the Environmental Health and Safety Division (including all those listed on page 2 of this document) are available to the public at no charge.

The safety issues identified in the consensus documents on the biology of specific crop plants are intended to address the potential for gene transfer within the crop plant species, and among related species, as well as the potential for weediness. They make no attempt to be definitive in this respect, however, as the many different environments in which the crop species may be grown are not considered individually.

This consensus document is a "snapshot" of current information that may be relevant in a regulatory risk assessment. It is meant to be useful not only to regulatory officials, as a general guide and reference source, but also to industry and others carrying out research.

In using this document, and others related to the biology of crop plants, reference to two OECD publications which have appeared in recent years will prove particularly useful. *Traditional Crop Breeding Practices: An Historical Review to Serve as a Baseline for Assessing the Role of Modern Biotechnology* presents information concerning 17 different crop plants. It includes sections on phytosanitary considerations in the movement of germplasm and on current end uses of the crop plants. There is also a detailed section on current breeding practices. *Safety Considerations for Biotechnology: Scale-Up of Crop Plants* provides a background on plant breeding, discusses scale dependency effects, and identifies various safety issues related to the release of plants with "novel traits."[2]

To ensure that scientific and technical developments are taken into account, OECD countries have agreed that consensus documents will be updated regularly. Additional areas relevant to the subject of each consensus document will be considered at the time of updating.

Users of this document are therefore invited to provide the OECD with new scientific and technical information, and to make proposals for additional areas to be considered. ***There is a short, pre-addressed questionnaire for that purpose at the end of this document. The completed questionnaire (or a photocopy) should be returned to the OECD's Environmental Health and Safety Division at the address shown.***

[2] For further information about these and other OECD publications which are on sale, contact the OECD's Publications Service, 2 rue André-Pascal, 75775 Paris Cedex 16, France. Fax: (33) 01 49 10 42 76. Internet email: PUBSINQ@ oecd.org

50

Section I – General Information

This consensus document addresses the biology of the potato (*Solanum tuberosum* subsp. *tuberosum*). It contains general information on the taxonomy, morphology, and centre of diversity of the species which can be of importance during a risk assessment (for example, information on reproductive biology, the possibility of crosses, and ecology). In regard to intra- and interspecific crosses, emphasis has been given to the conditions which make a cross possible rather than listing all successful crosses. Such a list would be very long and subject to frequent changes. Only hybridisation events not requiring human intervention are considered.

The Netherlands served as lead country in the preparation of this document, in collaboration with the United Kingdom.

Section II – Taxonomic Status

The family *Solanaceae* contains several well known cultivated crops such as tomato (*Lycopersicon esculentum*), eggplant (*Solanum melogena*), tobacco (*Nicotiana tabacum*), pepper (*Capsicum annuum*) and potato (*Solanum tuberosum*). The potato ranks, on a world scale, fourth in food production for starch crops. Around 300,000 metric tonnes are produced (FAO, 1985). About 95 countries produce potatoes, with a total value of around $US 13 billion (Horton et al., 1985).

Within the genus *Solanum* over a thousand species have been recognised. According to Burton (1989), there are "well over two thousand species". This genus is subdivided into several subsections, of which the subsection *potatoe* contains all tuber-bearing potatoes. The subsection *potatoe* is divided into series, one of which, *tuberosa*, is relevant to this document. Within the series *tuberosa* about 54 species, both wild and cultivated, are found. One of these is *S. tuberosum* (Hawkes, 1990).

S. tuberosum is divided into two subspecies: *tuberosum* and *andigena*. The subspecies *tuberosum* (**Table 1**) is the cultivated potato widely in use as a crop plant in, for example, North America and Europe. The subspecies *andigena* is also a cultivated species, but cultivation is restricted to Central and South America (Hawkes, 1990; Hanneman, 1994).

Table 1 Taxonomic position of *S. Tuberosum* subsp. *tuberosum*

Taxonomic rank	Latin name
family	Solanaceae
genus	*Solanum*
section	*petota*
subsection	*potatoe*
series	*tuberosa*
species	*Solanum tuberosum*
subspecies	*tuberosum*

Section III – Centre of Diversity

The centre of diversity for wild tuber-bearing potatoes (subsection *potatoe*) lies in Latin America, which is also considered the centre of origin. For the series *tuberosa* (to which *S. tuberosum* belongs) and most other series within the subsection *potatoe*, there are two centres of diversity. One is a long-stretching Andean area in Venezuela, Colombia, Ecuador, Peru, Bolivia and Argentina. The other is in central Mexico. The distribution area of these wild potatoes is much larger: from the southwestern United States to southern Argentina and Chile (Child, 1990; Hawkes, 1990).

Generally the cultivated *Solanum* species are also found within the centres of diversity for wild potatoes. The exception is the cultivated diploid form of *Solanum tuberosum* subsp. *tuberosum*, which is only found in a constricted area of southwestern Chile.

The cultivated tetraploid *Solanum tuberosum* subsp. *tuberosum*, as known in Europe and most other parts of the world, is considered to be a selection from a small introduction of *S. tuberosum* subsp. *andigena* potatoes from Colombia and Peru, and as such has a very narrow genetic basis. The arguments for this thesis are that plants of the original introductions into Europe are known to have been late flowering and tuberising, and that the morphological description of these potatoes matches the *andigena* type (Howard, 1970). Through selection, this introduction was adapted to the longer day lengths and different environmental conditions of Europe. Simmonds (1966) has shown that such transition can take place in a fairly short period of approximately ten years of selection. From Europe, this new type of potato has spread all over the world as a cultivated crop. An alternative theory is that, after the potato blight epidemic in Europe, new germplasm of *S. tuberosum* subsp. *tuberosum* originating from Chile (Hawkes, 1990) was introduced into Europe.

Section IV – Identification Methods

A. Morphology and Somaclonal Variation

The subsection *potatoe* is distinguished from all other subsections within the genus *Solanum* by "true potatoes whose tubers are borne on underground stolons, which are true stems, not roots" (Hawkes, 1994).

The series *tuberosa* is characterised by "imparipinnate or simple leaves, forked peduncle, rotate to petagonal corolla and round berries" (Hawkes, 1990). The species *S. tuberosum* is characterised by "pedicel articulation placed in the middle third, short calyx lobes arranged regularly, leaves often slightly arched, leaflets always ovate to lanceolate, about twice as long as broad, tubers with well marked dormancy period" (Hawkes, 1990).

The differences between the two subspecies within *S. tuberosum* are very small, the greatest difference being the short day dependence of the subspecies *andigena*. The differences are set out in **Table 2**.

Table 2 Distinction between *S. tuberosum* subsp. *tuberosum* and subsp. *andigena* (Hawkes, 1990)

Characteristic	*tuberosum*	*andigena*
leaves	less dissected	dissected
leaflets	wider	narrow
leaf angle	arched	acute
pedicel	thickened at the apex	not thickened at the apex
tuber formation	long or short days, mostly altitude neutral	short days, higher altitudes

The general description of the morphology of *S. tuberosum* subsp. *tuberosum* is as follows: Herbaceous perennial with weak stems that grow to a maximum of three feet, long pinnate leaves, ovate leaflets with smaller ones disposed along the midrib. The flowers are white, purple, pinkish, or bluish, in clusters, usually with a five-part corolla and exserted stamens with very short filaments. The fruits are yellowish or green, globose, and less than one inch in diameter. Some lack seeds, but others may contain several hundred. The fruits are inedible by humans due to the presence of toxins (Anonymous, 1996; Hawkes, 1990). Tubers are borne at the end of underground stolons. They are round to long oval. The flesh is generally white or cream to yellow, the skin colour light brownish to red. Tubers can contain high levels of solanine, a toxic alkaloid.

Potatoes are very easily regenerated with the use of *in vitro* tissue culture techniques. This form of vegetative propagation normally leads to genetically identical individuals, but considerable heterogeneity is common after tissue culture in which a callus stage is included. This variation is called somaclonal

variation. *S. tuberosum* subsp. *tuberosum* is, like all potatoes, quite prone to this kind of variation (Cutter, 1992; Hawkes, 1990).

B. Molecular Identification

It is also possible to distinguish between several *Solanum* species with the use of molecular techniques. Restriction Fragment Length Polymorphism (RFLP) of chloroplast DNA, using eight endonucleases (Hosaka et al., 1984), showed that 33 tuberous *Solanum* species and hybrids and two *Lycopersicon* species could be distinguished. The four different *Solanum tuberosum* subsp. *tuberosum* accessions showed identical patterns.

RFLP analyses of genomic DNA can also lead to species identification within the genus *Solanum*. Debener et al. (1990) showed with the use of 70 probe/enzyme combinations (probes from *S. tuberosum* subsp. *tuberosum*) that 38 accessions representing twelve *Solanum* species and one hybrid could be distinguished. The two accessions from *S. tuberosum* subsp. *tuberosum* were not identical. One of the two accessions was the "true" *S. tuberosum* subsp. *tuberosum* cultivar Bintje, the other was a breeding line in whose pedigree *S. andigena*, *S. demissum* and *S. acaule* were introgressed. It could also be shown that RFLP fingerprinting allows distinction not only between species but also between different cultivars or breeding lines (Weising et al., 1992). The use of probes from other *Solanum* species, mostly repetitive sequences, also led to species and cultivar identification (Schweizer et al., 1993). Also RAPD markers can be used for potato cultivar and clone fingerprinting (Powell et al., 1991; Quiros et al., 1993).

In addition, there are indications that the *Solanum* species can be distinguished with the use of the Amplified Fragment Length Polymorphism (AFLP) technique. One primer can generate up to 120 bands per sample, so that discrimination between *Solanum* species is very easy (Kardolus, in press).

It appears that the potato and tomato genomes are so preserved that probes from one can be used to identify the other (Gebhart et al., 1991). This is especially important for the construction of a genetic map and the development of molecular markers.

Section V – Genetic Characteristics: The Genome

The basic chromosome number in the genus *Solanum* is twelve. *S. tuberosum* subsp. *tuberosum* can be diploids (2n=24) or tetraploids (4n=48). The diploids are only found in Chile, while the tetraploids are the most commonly cultivated all over the world. How the tetraploidy originated is unclear. The cultivated *S. tuberosum* subsp. *tuberosum* can be either an autotetraploid (doubling of the chromosomes of a diploid species) or an allotetraploid (doubling of the chromosomes of a diploid hybrid between two related species) (Hawkes, 1990).

The phenomenon of unreduced gametes is common in *Solanum* species. In most *Solanum* species, next to the normal haploid gametes (n), unreduced gametes (2n) can be found, greatly extending the possible number of natural crosses (see section on crosses) (Hanneman, 1995). Watanabe et al. (1991) reported that most of the 38 tuber-bearing *Solanum* species examined produced 2n pollen. The frequency varied from 2 up to 10 per cent.

It is also fairly easy to produce dihaploids from cultivated tetraploid *S. tuberosum*. This can be done by pollinating with, for example, *S. phureja*, which leads to the formation of parthenogenetic diploid plants. Anther culture is also in use to produce dihaploids (Howard, 1970; Caligari,1992). It has been shown that, where *S. phureja* is used to produce dihaploids, minor chromosomal fragments are found in these dihaploids originating from *S. phureja* (Clulow et al., 1991).

The great value of these diploids is in breeding programmes: species that do not cross readily with the tetraploid potato can cross with a dihaploid (see section on crosses below). These dihaploids are often ovule fertile but pollen sterile.

Section VI – Reproductive Biology

A. Sexual Reproduction

Diploid *S. tuberosum* and the other diploid species within the section *petota* are self-incompatible (Kirch et al., 1989). This incompatibility is of a gametophytic, multi-allelic nature based on the occurrence of S alleles. In general these species are insect-pollinating, cross-breeding species

The cultivated tetraploid *S. tuberosum* subsp. *tuberosum* is self-compatible. The S alleles occur in this species, but somehow the incompatibility system is weakened. The mechanism behind this is not known. Plaisted (1980) has shown that under field conditions selfing is most likely for tetraploid *S. tuberosum*, with 80-100 per cent of the seeds formed due to selfing.

To facilitate cross-breeding and selfing, the appearance of insects is necessary. In particular, bumblebees (e.g. *Bombus funebris* in Peru and *B. impatiens* in the US) are good pollinators for potatoes (White, 1983). Pollen dispersal is mainly limited by the distance pollinating insects fly. Bumblebees and bees do not fly much further than three kilometres (Reheul, 1987). Normal honeybees (*Apis mellifera*) and *Bombus fervidus* are not pollinators of potato, as the flowers are without any nectar (Sanford and Hanneman, 1981). White (1983) carried out some experiments to determine the importance of pollination by wind for potatoes. Flowers were emasculated, and therefore of no interest to insects. The seedset on these flowers was assessed. No seeds were found, and therefore it was concluded that pollination by wind was of no importance.

Conner et al. (1996) collected outcrossing data from several field experiments with genetically modified potatoes, performed in New Zealand, the United Kingdom and Sweden. In each study the outcrossing rate was reduced to 0 per cent where the receiving plants were separated by more than 20 metres from the genetically modified ones.

Although many *Solanum* species are fertile, it appears that a large number of the tetraploid cultivated *S. tuberosum* subsp. *tuberosum* cultivars have a reduced fertility (Ross, 1986). Most cultivars show a reduced pollen fertility or even pollen sterility. Amongst them are well known cultivars like Bintje and King Edward. Although reduced female fertility is not so common, it is noticed that a lot of cultivars flower less profusely than wild material. Another observable phenomenon is that flowers are dropped after pollination, so that no berries are found. The result is that on most *S. tuberosum* subsp. *tuberosum* cultivars few berries and seeds are formed.

Potato seeds cannot be disseminated by birds, but dissemination by small mammals is possible (Hawkes, 1988). Lawson (1983) showed that in Scotland potato seeds could be stored in the ground for up to ten years without losing viability. Love et al., 1994 report that potato seeds can survive and germinate for periods of time in excess of seven years.

B. Asexual Reproduction

The potato can also multiplicate vegetatively. Tubers are formed under the ground. As the tuber is the product for which potatoes are cultivated, an extensive selection has taken place for good tuber production and quality. These tubers can remain viable for long periods of time as long as there is not a major frost period. The stolons on which the tubers are formed are generally not very long for *S. tuberosum* subsp. *tuberosum* cultivars. Stolons of wild tuber-bearing *Solanum* species are much longer (Hawkes, 1990).

Section VII – Crosses

A. Intraspecific Crosses

Solanum tuberosum subspecies *tuberosum* and *andigena* are fully cross-compatible (Plaisted, 1980). Hybrids can occur in nature. The incidence of this cross is not clear, as the morphological distinction between the two subspecies is very small. As both subspecies only occur in southern North America and some parts of South America, natural crosses are only likely to be found there.

B. Interspecific Crosses: Crosses within the Subsection *potatoe* (Tuber-bearing Potatoes)

The gene pool for potato is extremely large. Dale et al. (1992) and Evenhuis et al. (1991) state that it is likely that all crosses between the tuber-bearing potatoes within the section *petota* may be possible, although in some cases techniques will have to be applied to establish the crosses.

It appears that there are two groups within this section which are very difficult to cross:

- the diploid species in the series *morelliformia*, *bulbocastana*, *pinnatisecta*, *polyadenia*, *commersoniana*, *lignicaulia*, and *circaeifoli;*
- the diploid species in the other series.

The fertilization of a diploid plant with normal haploid pollen in fact consists of two fertilizations. The pollen contains two (haploid) generative nuclei; one nuclei fertilizes the egg cell, the other fertilizes the embryosac nucleus. The result is a diploid embryo with triploid endosperm.

Den Nijs and Peloquin (1977) reported the existence of a "triploid block" where a tetraploid plant was crossed with a diploid plant. This block is due to the imbalance between the endosperm (5x) and the embryo (3x). The endosperm is not formed, and this is followed by embryo abortion (Jacobsen and Rousselle, 1992).

Johnston et al. (1980, 1982) also found that some species of the same ploidy level could not cross, whereas crosses between species of different ploidy levels were successful. They introduced the concept of the Endosperm Balance Number (EBN), which is a measure to express the "effective ploidy of a genome in the endosperm". To make the normal development of the endosperm after fertilization possible, the maternal EBN must be twice the paternal EBN (2:1).

The EBN is independent of the ploidy level of the species, and its behaviour is additive. This means, for instance, that by doubling of the chromosome number the EBN also doubles.

Two situations can occur:

- the EBN of two species is the same: natural crosses are possible;

- the EBN of two species is not the same: natural crosses are not possible.

Where the EBN of two species is not the same, several natural or artificial mechanisms are available to circumvent the incompatibility.

Natural mechanisms:

- The occurrence of unreduced gametes makes it possible that species with a lower EBN can cross with species with a higher EBN. For example:

  A plant with 4x (EBN=4) cannot cross with a 2x (EBN=2) plant, but if the 2x plant produces unreduced gametes the EBN of these gametes becomes 4, which makes the cross possible. The resulting plant is a tetraploid (4x) with an EBN of 4. It is important to notice that, due to the common occurrence of unreduced gametes in most *Solanum* species, crosses of this kind can occur in nature.

Artificial mechanisms:

- Production of dihaploids makes it possible that species with a higher EBN can cross with species with a lower EBN. For example:

  A plant with 4x (EBN=4) cannot cross with a 2x (EBN=2) plant. After dihaploidisation of the 4x (EBN=4) plant a diploid plant with an effective EBN of 2 is formed. This plant can be pollinated by the 2x (EBN=2) plant. The resulting plant is a diploid (2x) with an EBN of 2. It is important to notice that crosses of this kind are not likely to occur in nature, due to the fact that diphaploids are only rarely formed in nature.

- Polyploidisation of plants makes it possible to cross plants with a lower EBN with plants with a higher EBN. For example:

  A plant with 2x (EBN=2) cannot cross with a 4x (EBN=4) plant. After polyploidisation of the 2x (EBN=2) plant a tetraploid plant with an effective EBN of 4 is formed. This plant can cross with the 4x (EBN=4) plant. The resulting plant is a tetraploid (4x) with an EBN of 4. It is important to notice that crosses of this kind are not likely to occur in nature, due to the fact that spontaneous polyploidisation rarely occurs; nevertheless, it cannot be excluded.

Despite the EBN system, potatoes of different groups can be combined by somatic fusion *in vitro*. The application frequency of this method is increasing. Fusion products may be fertile, so somatic hybrids may serve as a bridge for combining incompatible genomes.

In **Annex I** the ploidy and EBN of the most common potato species within the section *petota* are given. These data can be used as an indication of the possibility of formation of hybrids of *S. tuberosum* subsp. *tuberosum* with these species in nature. It is likely that *Solanum tuberosum* subsp. *tuberosum* can cross readily with all *Solanum* species mentioned in Annex I with the same EBN (=4). Also, due to the occurrence of unreduced gametes, the crosses of *S. tuberosum* subsp. *tuberosum* with all *Solanum* species mentioned in Annex I with an EBN of 2 are possible.

To determine if a cross really is likely to occur in nature, several factors have to be considered. The most important are:

- the EBN of the crossing partners:

  These must be matching, or the EBN of one partner must not be less than half the EBN of the other partner.

- geographical occurrence of the species involved:

  The species involved must occur in the same area and habitat.

- flowering period of the species involved:

  The flowering periods must overlap.

- the presence of stylar barriers that prevent the growth of pollen tubes:

  The presence of appropriate pollinators.

In most parts of the world, no *Solanum* species from the section *petota* with an EBN of 2 or 4 will occur next to cultivated tetraploid *S. tuberosum* subsp. *tuberosum*. Crosses are therefore not likely, due to geographical isolation. Only in the southern United States and South America do crossing partners with a suitable EBN occur next to cultivated tetraploid *S. tuberosum* subsp. *tuberosum*. In these areas the characteristics of the species involved and the habitat must be assessed to predict the likelihood of the cross.

C. Interspecific Crosses: Crosses with Species from Sections other than *petota*

The occurrence of hybrids with species from sections other than *petota* is not likely, due to strong crossing barriers, although in several areas of the world cultivated tetraploid *S. tuberosum* subsp. *tuberosum* occurs next to indigenous *Solanum* species (not from section *petota*). For instance, *S. nigrum* and *S. dulcamara* occur in the Netherlands. Eijlander and Stiekema (1990) found that the cross of tetraploid *S. tuberosum* subsp. *tuberosum* with *S. dulcamara* did not result in any viable seeds and plants. For the cross of *S. nigrum* with *S. tuberosum* the same is valid. Only after embryo rescue could two hybrids be obtained. These were less vital, male sterile, formed no tubers, and showed less female fertility. After pollination (backcross), no viable seeds were formed.

Section VIII – Ecology of *Solanum tuberosum* subsp. *tuberosum*

A. Cultivation

Environmental conditions under which *S. tuberosum* can be successfully grown are very diverse, as can be concluded from the fact that potatoes are cultivated in many parts of the world. A broad spectrum of cultivars are adapted to these different environmental conditions. Some general parameters can be determined for the cultivation of *S. tuberosum* subsp. *tuberosum*:

- The *S. tuberosum* subsp. *tuberosum* tuber cannot survive a temperature of -3°C and lower. The foliage dies at temperatures of -4°C (van Swaaij et al., 1987; Vayda, 1994). Dale (1992) reports that potato tubers are destroyed by a frost period of 25 hours at -2°C or a frost period of five hours at -10°C. Latin American *Solanum* species can be much more frost-resistant.

- *S. tuberosum* subsp. *tuberosum* cannot be acclimatised to lower temperatures, whereas other Solanum species (for example, S. acaule) can be (van Swaaij et al., 1987; Li and Fennel, 1985).

- Potatoes seem to be very sensitive to soil water deficit (Vayda, 1994).

- A wide range of soil pH can be tolerated by potatoes (normally 5 and higher, but good production at pH 3.7 was observed) (Vayda, 1994).

- S. tuberosum subsp. tuberosum is a daylight neutral crop, which means that tubers are made at a growth stage independent of the day length. But variation for daylight sensitivity can be found among *S. tuberosum* subsp. *tuberosum* cultivars.

- Extreme low or high temperatures, in particular the night temperature, can obstruct tuber formation.

- Short days (-14 hours) and moderate ground temperatures (15-18°) enhance tuber formation. Longer days (14-16 hours) and higher (day) temperatures (20-25°) enhance flowering and seed formation (Beukema and van der Zaag, 1979; Burton, 1989).

- Germplasm from all over the world has been used to improve potato varieties. The main goal is to develop cultivars with resistance to biotic factors (fungal, virus, bacterial and insect resistance). Other goals are: improved starch content, adaptation to tropical growing conditions, herbicide resistance, stress tolerance, and the introduction of anti-bruise genes (Brown, 1995). The species most used to improve potato are *S. demissum*, *S. acaule*, *S. chacoense*, *S. spegazinii*, *S. stoloniferum*, *S. vernei*. Less used are *S. microdontum*, *S. sparsipilum*, *S. verrucosum*, *S. phureja*, *S. tuberosum* subsp. *andigena*, *S. commersonii* and *S. maglia* (Caligari, 1992). This germplasm has been introduced into many cultivars of *S. tuberosum* subsp. *tuberosum*.

In **Annex II** the most common diseases (insects, mites, viruses, bacteria and fungi) in potato and their spread throughout the world are shown. This annex is not intended to give a complete list of all potato diseases known. Therefore it should be taken into account that locally other diseases can be of great

importance. The national phytosanitary service can best be consulted on this subject. Moreover, Annex II does not give any indication of which sanitary or quarantine provisions have to be applied in a country.

B. Volunteers and Weediness

In the cultivation of *S. tuberosum* subsp. *tuberosum*, plants from seeds from a previous potato crop can act as a volunteer weed. The tubers can also act as a volunteer weed in cultivation. In general these plants (from seeds and tubers) will be eliminated by normal agronomical practices. In addition, tubers will not survive for a long time in most of the areas of cultivation due to unfavourable environmental conditions (low temperatures).

Outside the field, potato seedlings will have difficulty establishing themselves as they cannot compete with other plants. Love et al., 1994 report that these seedlings are limited to cultivated areas for reasons of competition and adaptation. Potato tubers can be spread during transportation and use, but generally these plants will not be established for a long time due to unfavourable environmental conditions.

In general, the potato is not known as a coloniser of unmanaged ecosystems. In climax vegetation it is not able to compete with other species such as grasses, trees and shrubs (Anonymous, 1996).

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65

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Annex I

Ploidy and EBN of most *Solanum* species within the section *petota*
(Hawkes, 1990; 1992; 1994)

SUBSECT.	SERIES	PLOIDY	EBN=1	EBN=2	EBN=4	EBN=unknown
estolonifera	–	diploid	all species			
juglandifolia	–	diploid				all species
potatoe	*morelliformia*	diploid				*S. morelliforma*
	bulbocastana	diploid	*S. bulbocastanum*			*S. clarum*
		triploid	*S. bulbocastanum*			
	pinnatisecta	diploid	*S. branchistotrichum* *S. jamesii* *S. cardiophyllum* *S. pinnatisectum* *S. trifidum*			*S. tarnii*
		triploid	*S. cardiophyllum*			*S. jamesii*
	polyadenia	diploid				*S. polyadenium* *S. lesteri*
	commersoniana	diploid	*S. commersonii*			
		triploid	*S. commersonii*			*S. calvescens*
	circaeifolia	diploid	*S. capsicibaccatum* *S. circaeifolium*			
	lignicaulia	diploid	*S. lignicaule*			
	olmosiana	diploid				*S. olmosense*

68

SUBSECT.	SERIES	PLOIDY	EBN=1	EBN=2	EBN=4	EBN=unknown
	yungasensa	diploid		*S. chacoense* *S. tarijense*		*S. arnezii* *S. yungasense*
	megistacroloba	diploid		*S. astileyi* *S. megistacrolobum* *S. sanctae-rosae* *S. toralapanum*		*S. boliviense*
	cuneoalata	diploid		*S. infundibuliforme*		
	conicibaccata	diploid		*S. chomatophilum* *S. violacceimarmoratum* *S. agrimonifolium* *S. colombianum* *S. oxycarpum*		*S. santolalle*
		hexaploid			*S. moscopanum*	
	piurana	diploid				*S. piurae*
	ingifolia	diploid				*S. ingifolium*
	maglia	diploid				*S. maglia*
		triploid				*S. maglia*
	tuberosa	diploid		wild: *S. berthaultii* *S. brevicaule* *S. bukasovii* *S. canasense* *S. gourlayi* *S. kurtzianum* *S. leptophyes* *S. medians* *S. microdontum* *S. multidissectum* *S. multiinteruptum* *S. sparsipilum*		wild: *S. alandiae* *S. hondelmanni* *S. neocardenasii* *S. okadae* *S. oplocense*

69

SUBSECT.	SERIES	PLOIDY	EBN=1	EBN=2	EBN=4	EBN=unknown
	tuberosa (cont'd)	diploid (cont'd)		*S. spegazzinii* *S. vernei* *S. verrucosum* cultivated: *S. phureja* *S. stenotomum*		cultivated: *S. ajanhuiri*
		triploid				wild: *S. maglia* *S. microdontum* cultivated: *S.* × *chaucha* *S.* × *juzepczukii*
		tetraploid			wild: *S. gourlayi* *S. oplocense* *S. sucrense* cultivated: *S. tuberosum* subsp. *tuberosum* *S. tuberosum* subsp. *andigena*	
		pentaploid				*S.* × *curtilobum*
		hexaploid			*S. oploscense*	
	aucalia	tetraploid		*S. acaule*		
		hexaploid			*S. albicans*	
	longipedicellatu	triploid				*S.* × *vallis-mexci*
		tetraploid		*S. fenderi* *S. hjertingii* *S. papita* *S. polytrichon* *S. stoloniferum*		

70

SUBSECT.	SERIES	PLOIDY	EBN=1	EBN=2	EBN=4	EBN=unknown
	demissa	triploid				*S.* × *semidemissum*
		hexaploid			*S. brachycarpum* *S. demissum* *S. guerreroense* *S. hougasii* *S. iopetalum*	*S. scheckii*

Annex II

The most common diseases in *Solanum tuberosum* subsp. *tuberosum* and their distribution: for each pest/disease category a reference to a more extensive review is given (Hide and Lapwood, 1992; Evans and Trudgill, 1992; Raman and Radcliffe, 1992)

INSECT AND MITE PESTS (review: Hooker, 1986) **Organism**	**Occurrence**
Myzus persicae, Phthorimaea operculella, Agriotes spp.	Worldwide
Macrosiphum euphorbiae, Aphis fabae, Empoasca devastans, Heliothus armigera, Spodoptera exigua	Worldwide except Africa
Plusia orichalcea, Sthenaridea pulsilla, Psylloides plana, Epicauta hiriticornis, Anomala dimidiata, Phyllognathus dionysius, Melolontha spp., *Odontotermes obesus, Eremotermes* spp., *Alcidodes westermanni, Myllocerus subfasciatus, Pyralis farinalis, Nipaecoccus vastator*	Asia
Empoasca fabae, Paratrioza cockerelli, Hypolithus spp.	North America
Diabrotica sp., *Epicauta* spp., *Premnotrypes* spp., *Phylophaga* spp., *Scrobipalpula absoluta, Scrobipalpopis solanivora, Symmetri-schema plaeseosema, Feltia experta, Stenotycha* spp., *Copitarsia turbata, Bonthinus mainon, Phenacoccus grenadensis, Liriomyza* spp.	Central and South America
Shistocerca gregaria, Liriomyza trifolii	Africa
Henosepilachna sparsa, H. vigintisexpunctata, Austroasca virigrisea, Listroderes obliquus, Heteronychus arator, Cheiroplatys latipes, Graphognathus leucoloma	Australia
Aphis nasturtii, Limonius spp., *Ctenicera* spp., *Conodorus* spp.	North America and Europe
Aphis gossypii	Central and South America, Asia
Aulacorthum solani	North America, Europe and Africa
Leptinotarsa decemlineata	North America, Europe and Asia
Epitrix spp.	North, Central and South America
Epilachna spp., *Polyphagotarsonemus latus, Thrips palmi, Gryllotalpa africana*	Africa and Asia

72

NEMATODES (review: Hooker, 1986) **Organism**	**Occurrence**
Globodera rostochiensis, Globodera pallida	Worldwide
Meloidogyne hapla	North America and temperate areas in general
Meloidogyne chitwoodi	Northwest America and parts of Western Europe
Nacobbus aberrans	Peru and Bolivia
Pratylenchus penetrans, Trichodorus and *Paratrichodorus* spp.	North America and Europe
Ditylenchus destructor	North America, Western Europe and the former USSR
Ditylenchus dipsaci	Western Europe
VIRUSES (review: Valkonen, 1994) **Organism**	**Occurrence**
Virus X (mild mosaic), leaf roll virus, Virus Y (severe mosaic), Virus A (mild mosaic), Virus S	Worldwide
Virus M	Europe and North America
Tobacco rattle virus	Europe, North America, Brazil and Japan
Mop top virus	Western Europe and Peru
Yellow dwarf virus	North America
Spindle tuber "viroid"	North America, former USSR and South Africa
Witches' broom (mycoplasma)	Europe, North America, Australia, China
BACTERIA (review: Hooker, 1986) **Organism**	**Occurrence**
Clavibacter michiganensis var. *sepedonicus* (ring rot), *Erwinia carotovora* ssp. *atroseptica* and subsp. *Carotovora* (blackleg, soft rot)	Europe and North America
Erwinia chrysanthemi (blackleg, soft rot)	Tropics and sub-tropics
Streptomyces scabies (common scab)	Worldwide
Pseudomonas solanacearum (brown rot)	Tropics and warm temperate zones

FUNGI (review: Hooker, 1986) **Organism**	**Occurrence**
Alternaria solani (early blight), *Botrytis cinerea* (grey mould), *Collectotrichum coccodes* (black dot), *Helicobasidium purpureum* (violet root rot), *Phytophtora infestans* (late blight), *Pythium ultimun* (watery wound rot), *Rhizoctonia solani* (black scurf), *Spongospora subterranea* (powdery scrab), *Verticillium albo-atrum, V. dahliae* (wilt)	Worldwide
Angiosorus solani (potato smut)	Central and South America
Fusarium spp. (wilt)	North America
Fusarium solani var. *Coeruleum* (dry rot), *Phoma foveata* (gangrene)	Europe
Helminthosporium solani (silver scruf), *Sclerotinia sclerotiorium* (stalk break)	Europe and North America
Macrophomina phaseolina (charcoal rot)	North America and India
Polyscytalum pustulans (skin rot)	Northern Europe, North America, Australia
Synchytrium endobioticum (wart)	Europe, North and South America, South Africa and Asia
Phoma exigua var.*Exigua* (gangrene), *Phytophtora erythroseptica* (pink rot)	Europe, North America and Australia

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75

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76

- Main Page - Plant Biosafety Office
- Plant Health and Production Division
- The Regulation of Plants With Novel Traits in Canada
- Status of Regulated Plants with Novel Traits (PNTs) in Canada
- Import Permit Requirements for Plants with Novel Traits (including Transgenic Plants), and their Products (D-96-13)
- Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits (Dir94-08)
- Guidelines for the Environmental Release of Plants with Novel Traits within Confined Field Trials in Canada (Dir2000-07)
- Decision Documents
- Summary of Confined Field Trials
- Fee Submission Form
- Contacts

Canadian Food Inspection Agency,
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Regulatory Directive T-1-09-96 : The Biology of *Solanum tubersum* (L.) (Potato)

A companion document to the Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits

Table of Contents

A. General Information
  1. Background
  2. Scope
B. The Biology of Solanum tuberosum
  1. General Description, Use as a Crop Plant and Origin of Species
  2. Brief Outlook of Agronomic Practices for Potatoes
  3. The Reproductive Biology of S. tuberosum
  4. The Centres of Origin of the Species
  5. Cultivated S. tuberosum as a Volunteer Weed
  6. Summary of Ecology of S. tuberosum
C. The Close Relatives of Solanum tuberosum
  1. Inter-Species/Genus hybridization
  2. Potential for Introgression of Genetic Information from S. tuberosum into Relatives
  3. Occurrence of S. tuberosum in Canada
D. Potential Interactions of Solanum tuberosum with Other Life Forms
  1. Table 1. Potential Interactions of S. tuberosum with Other Life Forms During its Life Cycle
E. Acknowledgments
F. Bibliography

Part A. General Information

A1.0 Background

Since 1988, Agriculture and Agri-Food Canada (AAFC) has been regulating the field testing in Canada of agricultural and horticultural crop plants with novel traits (PNT's). "Plants with novel traits" are defined as a plant variety/genotype possessing characteristics that demonstrate neither familiarity nor substantial equivalence to those present in a distinct, stable population of a cultivated species in Canada and that have been intentionally selected, created or introduced into a population of that species through a specific genetic change. "Familiarity" is defined as the knowledge of the characteristics of a plant species and experience with the use of that plant species in Canada. "Substantial equivalence" is defined as the equivalence of a novel trait within a particular plant species, in terms of its specific use and safety to the environment and human health, to those in that same species, that are in use and generally considered as safe in Canada, based on valid scientific rationale.

The PNT's can either be derived from recombinant DNA technologies or from traditional plant breeding. Regulated field testing is necessary when the PNT's have traits of concern, i.e., the traits themselves, their presence in a particular plant species or their use are: (1) considered unfamiliar when compared with products already in the market; (2) not considered substantially equivalent to similar, familiar plant types already in use, and regarded as safe.

71

Before PNT's may be authorized for unconfined release, they must be assessed for environmental safety. Regulatory guidelines entitled: Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits have been developed to define criteria and information requirements that must be considered in the environmental assessment of PNT's to ensure environmental safety, in the absence of confinement conditions.

A2.0 Scope

The present document represents a companion document to the regulatory guidelines Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits. It is intended to provide background information on the biology of Solanum tuberosum (L.), its centres of origin, its related species and the potential for gene introgression from S. tuberosum into relatives, and details of the life forms with which it may interact.

Such species-specific information will serve as a guide for addressing some information requirements of Part D of the regulatory guidelines. Specifically, it will be used to determine whether there are significantly different/altered interactions with other life forms, resulting from the PNT's novel gene products, which could potentially cause the PNT to become a weed of agriculture, become invasive of natural habitats, or be otherwise harmful to the environment.

The conclusions drawn in this document about the biology of S. tuberosum only relate to plants of this species with no novel traits. Novel traits of concern might confer new characteristics to the plant, that could impact on the environment pursuant to their unconfined release.

Part B. The Biology of Solanum tuberosum

B1.0 General Description, Use as a Crop Plant and Origin of Species

The potato (Solanum tuberosum) belongs to the Solanaceae family. This family includes, among 2000 other species, the tomato (Lycopersicum esculentum), sweet pepper (Capsicum annuum), eggplant (Solanum Melongena var. esculentum), tobacco (Nicotiana tabacum), and petunia (Petunia hybrida). The genus Solanum is a polymorphous and largely tropical and subtropical genus containing more than 1000 species (Fernald, 1970).

The potato is one of the world's most important crops, following wheat, maize, and rice. It has weak stems that grow to a maximum of three feet, long pinnate leaves, ovate leaflets with smaller ones disposed along the midrib. The flowers are white, purple, pinkish, or bluish, in clusters, usually with a five parted corolla and exserted stamens with very short filaments. Some varieties are male sterile, suffer from abscission of flowers, and rarely produce fruits. The fruits are yellowish or green, globose, less than one inch in diameter, some lack seeds, but others may contain several hundred. The fruits are inedible to humans, due to the presence of toxins (Hortus Third, 1976). Solanum species have an initial chromosome number of 12, but polyploidy is prevalent in both wild and cultivated potatoes.

All potatoes cultivated in Canada belong to the species Solanum tuberosum, a species widely cultivated as an annual in temperate regions for its edible underground tubers. The tubers are utilized for food, feed and as seed propagules. In North America, the crop is grown primarily for human consumption and for seed. In some areas, starch is manufactured from surplus production and cullage. Starch manufactured from potatoes has a wide range of end uses. The most extensive use is for sizing and coating in the paper industry. In the food industry, starch may be used in the preparation of instant puddings, in the manufacture of ice cream, as an additive to breads and biscuits, as a thickener in soups and a variety of other preparations in home cooking. Potatoes utilized for food may be fresh or processed; the cultivars grown for processing are also acceptable for fresh consumption or table stock, while some table stock cultivars are not suitable for processing and are limited to the fresh market. Fresh potatoes are usually prepackaged in consumer packs or may be sold at retail in open displays. The single most important processed product is frozen french fried potatoes and there is a significant production of potato chips. The processed potato products are usually preserved by freezing, canning and dehydrating and are prepared in a variety of consumer products. Tubers used as livestock feed are usually cullage from fresh pack operations, surplus production or waste from processing plants.

Potatoes are grown in all provinces and territories of Canada. The surface area devoted to commercial potato production in 1995 was 144,700 hectares. The average yield for the same period was 26.27 tonnes per hectare, giving an average production of 3.78 million tonnes. On a regional basis, Canadian commercial production was distributed in 1995 as follows: New

Foundland, Nova Scotia, New Brunswick and Prince Edward Island, 50%; Ontario and Quebec, 24%; and Manitoba, Saskatchewan, Alberta and British Columbia, 26%. Production is expanding rapidly in Prince Edward Island, and to some extent in Manitoba and Alberta. Production has remained relatively constant in all other provinces.

B2.0 Brief Outlook of Agronomic Practices for the Potato (from Hortus Third, 1976)

Cold summer temperatures are ideal for potato production. It is also essential to have ample soil moisture for optimum yields. Tuber formation in potatoes is favoured by short days. The potato does not breed true, and seeds may differ genetically in the same fruit and from the parent plant. Therefore, the seeds are of value only to the plant breeder who wishes to develop new genotypes. Ordinary propagation is through the planting of tubers or pieces of tubers presenting at least one eye. These propagules are known in the industry as "seed", not to be confused with true or botanical seed. Each eye of a potato tuber is made up of a rudimentary scale leaf and a multiple bud cluster. For each row of cultivated potatoes, the soil is ridged up to prevent exposure of the developing tubers to light. Tubers exposed to the light turn green and produce an antinutritional alkaloid, which renders them unfit for human or livestock consumption. For the same reason, potatoes should be stored in a dark place after harvest.

High application rates of nitrogen, phosphorus and potash are required for best yields in most locations, and magnesium is frequently needed in acid soils. Potatoes may be planted before the usual date of the last killing frost. Although the foliage is only moderately tolerant of frosts, new shoots will emerge if the seed pieces are still sound at the time of any frost damage.

There are many serious diseases that may be carried in seed potatoes, such as scab, a disease which causes corky lesions on the skin of the potato. Potatoes can also be attacked by such diseases as late blight and early blight, bacterial ring rot and virus diseases. The only protection against such diseases is to use certified disease free seed potatoes. A number of insects can attack the developing plant. Perhaps the most serious of these is the Colorado potato beetle, which can cause extensive foliar damage. Other insects attacking foliage include potato flea beetles, leaf hoppers, and aphids.

B3.0 The Reproductive Biology of S. tuberosum

The cultivated potato is an annual and may be reproduced by seed although the commercial crop is propagated from tuber «seed» pieces (Rowe, 1993). In the context of true seed production, the degree to which flowering occurs, the duration of flowering and the response of flowering behaviour to environmental conditions is greatly influenced by cultivar (Burton, 1989). The environmental conditions that influence flower initiation and development include light intensity, quality and duration (day length), temperature, water supply and available soil nutrients. Flower development does not ensure fruit set. Pollen sterility is frequently encountered, in which case pollinating insects may not be attracted due to poor nectar production, and ovule sterility is also occasionally experienced.

True seed production is practised in breeding programs, and under greenhouse conditions. It requires skilled manipulation of variables to be successful. True seed production in the natural environment varies with cultivars and weather conditions. Many varieties do not produce any seed, while some varieties are either self-pollinated or cross-pollinated and do produce seeds.

B4.0 The Centres of Origin of the Species

The potato originates from regions of high elevation of South America. The area of first domestication is reasoned to be the area where wild diploids are still found and where the greatest diversity of cultivated forms can still be found, and is identified as the high plateau of Bolivia and Peru, in the general region of Lake Titicaca (Hoopes and Plaisted, 1987).

Many authors have traced the origin and distribution of the potato (Burton, 1989; Dodds, 1965; Hawkes, 1967; Howard, 1970; Salaman, 1949; Simmonds, 1976). These authors essentially agree that the significant species considered to be the progenitors of the cultivated potato are from South America. Simmonds (1976) indicates these may be found to 450S latitude in South America and to 400N in North America.

Yield and quality enhancement have marked the changes that emerged with the introduction of plant breeding programs in Europe and North America in the 19th century. The need for pest resistance was introduced with the late blight (Phytophthora infestans) infestation of the 1840's, and, in the 20th century, a need for resistance to viruses and nematodes. This resistance was

attempted through the use of S. demissum for late blight, S. stoloniferum, S. chacoense and S. acaule for viruses and S. multidissectum, S. spegazzinii, S. kurtzianum, S. oplocense and S. vernei for nematodes. These wild species have been used extensively in breeding programs (Burton, 1989; De Jong and Tarn, 1984; Howard, 1970; Simmonds, 1976).

B5.0 Cultivated S. tuberosum as a Volunteer Weed

Some varieties of the cultivated potato produce true seed. In Southern England, this seed can survive for up to seven years (Lawson, 1983). In Canada, most production areas experience deep frost penetration in the soil profile and tubers that set on plants produced from true seed are regularly purged from the soil environment. In addition, plantlets that grow from true seed are easily controlled with normal weed control practices and do not usually set tubers. Thus, in land areas where potatoes are grown commercially, the measures required to control volunteers that result from true seed being dispersed do not differ from the cultural and pest management practices that are normally followed for the crop and succeeding crops in the rotation. Outside of cultivated areas, seedlings grown from true seed do not compete successfully and are not reported as a weed pest in these areas.

Vegetative volunteers may result from tubers that are left at harvest time. Plants established from such tubers have failed to survive outside of cultivated areas. In cultivated areas, they may become a short-term weed problem, but a combination of weed control efforts and unfavourable environmental conditions limits weediness to one or a few years only (Makepeace et al., 1978). The possibility for vegetative escape of tubers, however, is present in the handling and transportation of potatoes. Even though volunteers occur periodically near animal feed lots, at waste disposal sites and in areas surrounding commercial production sites, there is no evidence that potato plants will proliferate and become established as a weed. Evenhuis and Zadoks (1991), have concluded that this is the case because S. tuberosum is at a competitive disadvantage and does not survive in the wild.]

B6.0 Summary of Ecology of S. tuberosum

Solanum tuberosum is not a primary colonizer in unmanaged ecosystems. Seedlings of this species do not compete successfully against plants of similar type for space. In climax ecologies they are displaced by grasses on prairies or tree species and perennial shrubs in forests and, in Canada, do not survive outside of cultivation for significant periods of time.

In crop production systems, volunteer tubers and plants are removed with the production practices that are normally used for potatoes and the crops that succeed potatoes in the rotation.

Solanum tuberosum is not listed as a noxious weed in the Weed Seed Order (1986). It is not reported as a pest or weed in managed ecosystems in Canada, nor is it recorded as being invasive of natural ecosystems. In summary, there is no evidence that in Canada S. tuberosum has weed or pest characteristics.

Part C. The Close Relatives of Solanum tuberosum

C1.0 Inter-Species/Genus Hybridization

Important in considering the potential environmental impact following the unconfined release of genetically modified S. tuberosum, is an understanding of the possible development of hybrids through interspecific and intergeneric crosses with the crop and related species. The development of hybrids could result in the introgression of the novel traits into these related species and resulting in:

- the related species becoming more weedy
- the introduction of a novel trait with potential for ecosystem disruption into the related species.

This section will be subject to updating, as more data becomes available. Based on background information provided in the present document, applicants will need to consider the environmental impacts of potential gene flow.

For a trait to become incorporated into a species genome, recurrent backcrossing of plants of that species by the hybrid intermediaries, and survival and fertility of the resulting offspring, is necessary.

C2.0 Potential for Introgression of Genetic Information from S. tuberosum into Relatives

Less than a tenth of the Solanum species are tuber-forming and, so far as is known, these occur only in the Americas, between mid-USA to the north and southern Chile and Argentina to the south (Burton, 1989). Solanum fendleri and S. jamesii, found in the southern part of the US, can produce hybrids with S. tuberosum plants under controlled laboratory conditions (Adiwilaga and Brown, 1991). These species occupy dry forest at high altitudes, and therefore are quite unlikely to cross with cultivated potato plants under natural conditions due to geographical separation.

As a general rule, Solanum tuberosum can only hybridize naturally with Solanum species that form tubers (Dale et al., 1992; Evenhuis and Zadoks, 1991), none of which are present in Canada (Love and Pavek, 1994). Gene introgression to related native species through hybridization is therefore not possible (Love and Pavek, 1994 and other authors cited previously).

C3.0 Occurrence of S. tuberosum in Canada

The occurrence of S. tuberosum is limited to commercial production for harvest of tubers. Occasionally, plants and seedlings grow as volunteers from escape tubers and true seeds, but these do not become established in Canada as persistent weeds. Solanum tuberosum is not reported as a weed in Canada.

Part D. Potential Interactions of Solanum tuberosum with Other Life Forms

Table 1 is intended to be used to guide applicants in their considerations of potential impacts of the release of the PNT on non-target organisms.

The intention is not to require comparison data between the PNT and its S. tuberosum counterpart(s) for all interactions. Depending on the novel traits, applicants might decide to submit data for only some of the interactions. Sound scientific rationale will be required to justify the decision that data would be inappropriate or irrelevant for the remaining interactions. For example, the applicant might chose not to provide data on the weediness potential of the PNT if it can be clearly shown that the novel trait will not affect reproductive or survival characteristics of S. tuberosum, either directly or indirectly.

Some of the life forms are listed as categories (i.e., pollinators, mychorrhizal fungi, animal browsers, birds, soil microbes, and soil insects). When, because of the novel traits, a concern is perceived for these specific categories, applicants will be required to provide detailed information on interactions with indicator species in each category.

Where the impact of the PNT on another life form (target or non-target organism) is significant, secondary effects may need to be considered.

Table 1. Examples of potential interactions of Solanum tuberosum with other life forms during its life cycle.
(information requirements may be waived if valid scientific rationale is provided).

81

Other life forms Common Name	**Interaction with S. tuberosum Pathogen; Symbiont or Beneficial Organism; Consumer; Gene Transfer**
Bacterial ring rot (Clavibacter michiganense)	Pathogen
Black leg (Erwinia carotovora)	Pathogen
Common scab (Streptomyces scabies)	Pathogen
Early Blight (Alternaria alternata and A. solani)	Pathogen
Gray Mold (Botrytis cinerea)	Pathogen
Late Blight (Phytophthora infestans)	Pathogen
Leak (Pythium ultimum)	Pathogen
Leaf Roll Virus	Pathogen
Mosaic Virus	Pathogen
Spindle Tuber Viroid	Pathogen
Colorado Potato Beetle (Leptinotarsa decemlineata)	Consumer
Aphids (Myzus persica, Aphis nasturtii, Macrosiphum euphorbiae)	Consumer
Wireworm (Elateridae)	Consumer
Potato flea beetle (Epitrix cucumeris)	Consumer
Pollinators	Symbiont or Beneficial Organism; Consumer
Mychorrhizal fungi	Symbiont or Beneficial Organism; Consumer
Birds	Consumer
Animal browsers	Consumer
Soil microbes	Symbiont or Beneficial Organism
Earthworms	Symbiont or Beneficial Organism
Soil insects	Consumer
Other S. tuberosum	Gene transfer
Others	---

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83

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[Top of page]

January 3, 1996

Top of Page Important Notices

84

Potato

Importance of Potato

The potato (Solanum tuberosum L.) is a major world food crop. Potato is exceeded only by wheat, rice, and maize in world production for human consumption (Ross, 1986). Potato tubers give an exceptionally high yield per acre, many times that of any grain crop (Burton, 1969), and are used in a wide variety of table, processed, livestock feed, and industrial uses (Feustel, 1987; Talburt, 1987). Solanum tuberosum cv. Russet Burbank is the most popular potato cultivar in the United States.

Taxonomy of Potato

Potato belongs to the Solanaceae, a family of about 90 genera and 2,800 species. Although the family is found throughout the world, it is especially concentrated in the tropical regions of Latin America (Correll, 1962). The genus Solanum, to which potato and all wild relatives belong, consists of about 2,000 species. Within this genus, the section Tuberarium (Correll, 1962), also known as section Petota (D'Arcy, 1972), includes the tuber-bearing members, of which the cultivated potato is best known. The wild species of the section Tuberarium, numbering about 180, are prominent in the Peruvian and Bolivian Andes; they have been subject to repeated germplasm collecting expeditions, and still represent a rich source of diversity in breeding programs (Correll, 1962; Ross, 1986).

External Morphology of Potato

The potato is an herbaceous plant, 0.5-1 meter high. The leaves are alternate and irregularly pinnately compound. Inflorescences consist of several flowers. Flowers are 5-merous, actinomorphic, perfect, and have sympetalous colored corollas. Fruits are berries, absent in many cultivars (Burbank, 1921), and bicarpellate. Tubers form underground from rhizomes, from which adventitious roots are developed to become a fibrous mass (Burton, 1969).

Reproduction and Genetics of Potato

Potato genetics and reproduction is a complex field. It is difficult to do justice to the subject in a short exposition. Nonetheless, the following five points outline the subject and the pertinent matters as they relate to this EA.

- The potato has the richest genetic resources of any cultivated plant, and these genetic resources are generally easily incorporated into cultivars. The variation includes not only wild potato species in Andean South America, but also semi-cultivated plants, local land races, and hybrid swarms of cultivated and wild plants (Ross 1986).

- The potato has a series of ploidy levels, based on a haploid number of 12, ranging from diploid (2n=24) to hexaploid (6n=72), and including triploids, tetraploids, and pentaploids (Dodds, 1962). The cultivated potatoes are autotetraploid (4n=48); many wild species are diploid, but may range up to hexaploid. The tetraploid cultivated potatoes are not diploidized, so that there are four interchangeable genes at each locus (Ross, 1986). Genetic manipulation and peculiarities in the cultivars and breeding stock is extensive.

- The potato "seed" of commerce is not true botanical seed, but rather consists of sections of potato tuber with one or more "eyes", i.e. lateral buds (Everett, 1981). The potatoes of commerce are therefore all reproduced vegetatively, as clones. This necessarily means that once a cultivar is produced, it is genetically stable in perpetuity, barring mutation, clonal variation (Shepard et al.,

85

1980) or some other unusual event. It also means that potato clones are especially susceptible to disease transmission via the tuber sections (Ross, 1986).

- True potato seed (TPS), is genuine botanical seed of the potato, and once was the province of the plant breeder's art. It is now used sometimes in commercial and garden (Page, 1982; Page, 1985; Park, 1989) culture. There are several advantages to TPS, including prevention of disease transmission, storage and shipment convenience, and reduction of acreage used for seed production (Ross, 1986).

- Potato plants are notorious for sterility, both male and female (Ross, 1986). This causes difficulties in potato breeding. Most commercial cultivars are sterile (Burbank, 1921). Russet Burbank is a male sterile cultivar. Russet Burbank potatoes will not produce viable pollen that will fertilize potato flowers and thus spread genetic material. Lemhi Russet potatoes, as documented in this submission, is capable of self-pollination, and thus could produce viable seed.

Cultivation and Use of Potato in the United States

Potatoes are grown commercially in the United States as monoculture rowcrops of a single clone. Tuber sections of the selected cultivar are planted, treated with fertilizer, treated with pesticides, and mechanically harvested (Burton, 1969). About 7 percent of the tubers of each year are used as seed; about 48 percent are processed; and about 30 percent are used as fresh vegetables (Talburt, 1987).

Distribution of Cultivated Potatoes

The aboriginal home of cultivated potatoes, in the South American Andes, still possesses a wide range of wild potatoes, cultivated potatoes, and hybrid swarms of intermediate potatoes at various ploidy levels. But that is not the major world center of potato culture. Most potatoes are grown in temperate climates or the mountains of tropical areas. The major world producers, in order of production, are U.S.S.R, Poland, United States, East Germany, West Germany, and France (Talburt, 1987).

In the United States, potatoes are widely grown, but especially in the States of Idaho, Washington, Oregon, Colorado, North Dakota, Wisconsin, and Maine (Jewell, 1988).

Sexually Compatible Potato Relatives in the United States

Potentially compatible relatives of the Russet Burbank and Lemhi Russet potato cultivars in the United States are the other cultivars of the potato, Solanum tuberosum, and a few wild members of Solanum section Tuberarium that occur in the southwestern United States (Correll, 1962). However, because of male sterility in Russet Burbank potatoes, no actual sexual compatibility exists for this cultivar. The cultivar Lemhi Russet does produce pollen, but documentation in this submission indicates that cross pollination from the experimental plants to other potato plants will not occur.

Modes of Gene Escape in Russet Burbank and Lemhi Russet

In Russet Burbank potatoes, gene escape by pollen is precluded because of male sterility. In Lemhi Russet potatoes gene escape by pollen is unlikely unless sexually compatible relatives are in the immediate proximity of the test area. According to documentation in the submission, transfer by insect pollinators is unlikely even in this instance.

Gene escape by seed is not possible in Russet Burbank, because this cultivar will not produce seed. Seed from self pollination may be produced by the cultivar Lemhi Russet, and positive steps must be taken to prevent escape of these seeds.

Gene escape could occur by the mechanical removal of potato tubers from the site. Precautions are necessary to either prevent the removal of such tubers, or to ensure the natural or artificial destruction of

86

such tubers.

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Crops No Longer Regulated

87

Biotechnology Permits Home Page

88

Annex 2

Joint USDA/CFIA Molecular Characterization and Data Standards

APPENDIX I:
MOLECULAR GENETIC CHARACTERIZATION DATA

INTRODUCTION

In July of 1998, regulatory officials of the Canadian Food Inspection Agency (CFIA), Health Canada, and the United States Department of Agriculture's Animal and Plant Health Inspection Service (USDA-APHIS) met to compare, and harmonize where possible, aspects of molecular genetic characterization that are part of their review processes for transgenic plants. Agreement on common requirements and acceptable analytical approaches for molecular genetic characterization will facilitate the submission of supporting data by developers seeking regulatory approval to incorporate such plants into agricultural production or commerce in both countries. This Appendix is one of the outcomes of this meeting. The Appendix summarizes and identifies similarities and differences in the critical elements of the molecular genetic characterization of transgenic plants considered during the review process by these participating agencies. Molecular genetic characterization is only part of the information considered during assessments of such plants conducted prior to commercialization.

The scope of this document is limited to consideration of the transformation process and vectors used during transformation; the genetic material that was potentially delivered to the recipient plant; the identification, inheritance, and expression of the genetic material in the transgenic plant, and the production of new proteins encoded by the introduced genetic material. This document does not address specific types of techniques nor quality assurance practices (e.g., good laboratory practices) that are used to generate molecular genetic characterization data.

The agencies found very substantial areas of agreement in the types of molecular genetic characterization data they require to be submitted and considered. In addition to the specific data sets reviewed, the participants of both countries reaffirmed that reviews are still conducted on a case-by-case basis which allows for reviewing additional or fewer data sets, depending upon the individual case and the regulatory authority of the individual agencies. The use of the word "may" in this document is intended to reflect some of this flexibility in determining when data sets will be considered as an appropriate part of the entire application package. Therefore, consultations between regulatory agencies and individual applicants are considered to be an important part of the overall application process in making such determinations.

The critical elements of the molecular genetic characterization of transgenic plants described below apply to the review process of the participating agencies in both Canada and the United States, except where noted. The contents of this document will be reviewed and amended as necessary by these agencies. The glossary which follows has been included to provide definition to certain terms within the context of this document.

GLOSSARY

carrier DNA DNA used to expedite the preparation or the transformation of genetic material into a plant but which is itself not part of the construct.

coding region A DNA sequence which can be translated to produce a protein. Synonymous with open reading frame.

construct An engineered DNA fragment (eg. plasmid) which contains, but is not limited to, the DNA sequences to be integrated into a target plant's genome.

database citations Publicly accessible sources of nucleotide or protein sequence information. Four commonly used databases and their website addresses are:

> **GenBank**: An annotated collection of all publicly available DNA sequences maintained by the National Institute of Health (NIH). http://www.ncbi.nlm.nih.gov/Genbank/GenbankOverview.html
>
> **DNA Data Bank of Japan**: The officially certified DNA bank of Japan, which collects DNA sequences from researchers. http://www.ddbj.nig.ac.jp/fromddbj-e.html
>
> **EMBL Nucleotide Sequence**: A database of DNA and RNA sequences collected from the scientific literature, patent applications, and directly submitted from researchers and sequencing groups. http://www.ebi.ac.uk/ebi_docs/embl_db/ebi/topembl.html
>
> The **SWISS-PROT Protein Sequence Data Bank**: A database of protein sequences produced collaboratively by Amos Bairoch (University of Geneva) and the EBI. http://www.ebi.ac.uk/swissprot/

insert That part of a construct (see above) which is integrated into the recipient plant's genome.

non-coding region DNA sequences which lie outside of an open reading frame and which are not translated to become part of a protein. These might include scaffold attachment regions, promoters, leader sequences, enhancers, introns, terminators, and any other sequences that are used for gene expression either in the plant or other hosts. such as origins of replication, transposable elements, T-DNA borders, *lox* sequences, etc..

stability	The ability of the transgenic trait to be expressed in the transformed plant line and plant lines derived therefrom in a consistent, reliable, and predictable manner.
trait(s)	The phenotypic characteristic(s) conferred to the recipient plant by the transgenic insert.
vector	An automonously replicating DNA molecule into which foreign DNA is inserted and then propagated in a host cell.

MOLECULAR GENETIC CHARACTERIZATION OF TRANSGENIC PLANTS

1 THE TRANSFORMATION SYSTEM

1.1 Description of the transformation method

1.1.1 Describe and provide references for the transformation method, e.g. *Agrobacterium*-mediated transformation or direct transformation by methods such as particle bombardment, electroporation, PEG transformation of protoplasts, etc.

1.1.2 For direct transformation methods, describe the nature and source of any carrier DNA used.

1.1.3 For *Agrobacterium*-mediated transformation, provide the strain designation of the *Agrobacterium* used during the transformation process, and indicate how the Ti plasmid based vector was disarmed, and whether *Agrobacterium* was cleared from the transformed tissue.

1.1.4 For transformation systems other than *Agrobacterium*, provide the following information:

1.1.4.1 Does the system utilize a pathogenic organism or nucleic acid sequences from a pathogen?

1.1.4.2 How were any pathogenesis-related sequences removed prior to transformation?

1.1.4.3 Did the transformation process involve the use of helper plasmids or a mixture of plasmids? If so, describe these in detail.

1.2 Description of the genetic material potentially delivered to the recipient plant material (the modification/constructs).

1.2.1 Provide a summary of all genetic components which comprise the vector including coding regions, and non-coding sequences of known function (see Table 1). For each genetic component provide a citation where these functional sequences were described, isolated, and characterized (publicly available database citations are acceptable) and indicate:

1.2.1.1 The portion and size of the sequence inserted.

1.2.1.2 The location, order, and orientation in the vector.

1.2.1.3 The function in the plant.

1.2.1.4 The source (scientific and common, or trade name, of the donor organism).

1.2.1.5 If the genetic component is responsible for disease or injury to plants or other organisms, and is a known toxicant, allergen, pathogenicity factor, or irritant.

1.2.1.6 If the donor organism is responsible for any disease or injury to plants or other organisms, produces toxicants, allergens or irritants or is related to organisms that do.

1.2.1.7 If there is a history of safe use of the source organism or components thereof.

1.2.2 If there has been a significant modification that affects the amino acid sequence of genes designed to be expressed in the plant, provide the citation. If the modified amino acid sequence has not been published, provide the complete sequence highlighting the modifications. Modifications that affect only a few amino acids can simply be stated without providing the complete sequence. Indicate whether the modifications are known or expected to result in changes in post-translational modifications or sites critical to the structure or function of the gene product.

1.2.3 Provide a detailed map of the vector (see Figure 1) with the location of sequences described above that is sufficient to be used in the analysis of data supporting the characterization of the DNA, including as appropriate the location of restriction sites and/or primers used for PCR and regions used as probes.

2 INHERITANCE AND STABILITY OF INTRODUCED TRAITS WHICH ARE FUNCTIONAL IN THE PLANT

2.1 For plants which are either male or female fertile or both, provide data that demonstrates the pattern and stability of inheritance and expression of the new transgene traits. If the new trait can not be directly measured by an assay, it may be necessary to examine the inheritance of the DNA insert directly, and expression of the RNA.

2.2 For plants which are either infertile or for which it is difficult to produce seed (such as vegetatively propagated male-sterile potatoes), provide data to demonstrate that the transgene trait is stably maintained and expressed during vegetative propagation over a number of cycles that is appropriate to the crop.

3 CHARACTERIZATION OF THE DNA INSERTED IN THE PLANT

3.1 For <u>all</u> coding regions, provide data that demonstrate if complete or partial copies are inserted into the plant's genome. Coding regions may include truncated sense constructs,

sequences engineered to be nontranslatable, antisense constructs, and constructs containing ribozymes, regardless of whether or not the coding region is designed or expected to be expressed in the transgenic plant. For Canadian submissions, information may be required indicating the number of copies which have been inserted, including integration of partial copies; and for allopolyploid plants, information indicating into which parental genome insertion has occurred.

3.2 For noncoding regions associated with the expression of coding regions:

3.2.1 Data should demonstrate whether or not plant promoters are inserted intact with the coding regions whose expression they are designed to regulate.

3.2.2 DNA analysis may be necessary for introns, leader sequences, terminators, and enhancers of plant-expressible cassettes.

3.2.3 DNA analysis may be necessary for promoters and other regulatory regions associated with bacteria-expressible cassettes.

3.3 For noncoding regions which have no known plant function and are not associated with expression of coding regions:

3.3.1 DNA analysis may be required for some sequences of known function (e.g., *ori V* and *ori-322*, *bom*, T-DNA borders of *Agrobacterium*, and bacterial transposable elements).

3.3.2 DNA analysis is not required for any remaining sequences of the plasmid backbone.

4 PROTEIN AND RNA CHARACTERIZATION AND EXPRESSION

4.1 For all complete coding regions inserted, provide data that demonstrates whether the protein is or is not produced as expected in the appropriate tissues consistent with the associated regulatory sequences driving its expression (e.g., if the gene is inducible, determine if the gene is expressed in the appropriate tissues under induction conditions). For virus resistant plants where the transgenes are derived from a viral genome, in addition to transgene protein analysis, determine transgene RNA levels in tissues consistent with the associated regulatory regions driving expression of the transgene. The following exceptions also apply:

4.1.1 If the protein concentration is below the limits of detection, mRNA data may be substituted.

4.1.2 Protein analysis for products of genes used only as selectable markers may be waived under certain circumstances, e.g. when there is at least one complete copy of a selectable

95

marker gene present and the effective expression of the selectable marker gene is verified by the process used to select the transformed tissue.

4.1.3 For plants modified to express nontranslatable mRNA, truncated sense constructs, antisense constructs, or constructs containing ribozymes, since the function of these genetic constructs is to specifically alter the accumulation of a specific mRNA or protein present in the transgenic plant, provide data on the level of the target protein only (e.g. native tomato fruit polygalacturonase would be the target protein of antisense polygalacturonase to achieve altered fruit ripening). If the target protein levels are below levels of detection, determine target mRNA levels.

4.2 When a fragment of a coding region designed to be expressed in a plant is detected, determine whether a fusion protein could be produced and in which tissues it may be located.

4.3 Protein or RNA characterization may not be required for fragments of genetic constructs not expected to be functional in the plant (e.g., fragments of selectable marker genes driven by bacterial promoters.)

Table 1: Example of a Table Describing the DNA Components of a Vector

Summary of DNA Components in PV-STBT02 (from Table III.1 from APHIS petition # 94-257-01p)

Genetic Element	Size[1], Kb	Function and Source
RB	0.36	A restriction fragment from the pTiT37 plasmid containing the 24 bp nopaline-type T-DNA right border used to initiate the T-DNA transfer from *Agrobacterium tumefaciens* to the plant genome (Depicker *et al.*, 1982)
E35S	0.62	The cauliflower mosaic virus (CaMV) promoter (Odell *et al.*, 1985) with the duplicated enhancer region (Kay *et al.*, 1987).
cryIIIA	1.8	The gene which confers resistance to CPB. The gene encodes an amino acid sequence identical to the CPB control protein (referred to as the *B.t.t.* Band 3 protein) found in *B.t.t.* as described by Perlak *et al.* (1993).
E9 3'	0.63	A 3' nontranslated region of the pea ribulose-1,5-bisphosphate carboxylase small subunit (rbcS) E9 gene (Coruzzi *et al.*, 1984), which functions to terminate transcription and direct polyadenylation of the *cryIIIA* mRNA.
NOS 3'	0.26	A 3' nontranslated region of the nopaline synthase gene which functions to terminate transcription and direct polyadenylation of the *nptII* mRNA (Depicker *et al.*, 1982; Bevan *et al.*, 1983).
nptII	0.79	The gene isolated from Tn5 (Beck *et al.*, 1982) which encodes for neomycin phosphotransferase type II. Expression of this gene in plant cells confers resistance to kanamycin and serves as a selectable marker for transformation (Fraley *et al.*, 1983).
35S	0.32	The 35S promoter region of the cauliflower mosaic virus (CaMV) (Gardner *et al.*, 1981; Sanders *et al.*, 1987).
LB	0.45	A restriction fragment from the octopine Ti plasmid, pTi15955, containing the 24 bp T-DNA left border used to terminate the transfer of the T-DNA from *Agrobacterium tumefaciens* to the plant genome (Barker *et al.*, 1983).
ori V	1.3	Origin of replication segment for ABI *Agrobacterium* derived from the broad-host range plasmid RK2 (Stalker *et al.*, 1981).
ori-322/rop	1.8	A segment of pBR322 which provides the origin of replication for maintenance of the PV-STBT02 plasmid in *E. coli*, the replication of primer (*rop*) region and the *bom* site for the conjugational transfer into the *Agrobacterium tumefaciens* cells (Bolivar *et al.*, 1977; Sutcliffe, 1978).
aad	0.93	A fragment isolated from transposon Tn7 containing a 0.79 kb gene which encodes for the enzyme streptomycin adenylyltransferase that allows for bacterial selection on spectinomycin or streptomycin (Fling *et al.*, 1985).

1. Sizes are approximations.

Figure 1: Example of a detailed map of a plasmid vector (from APHIS petition #94-257-01p).

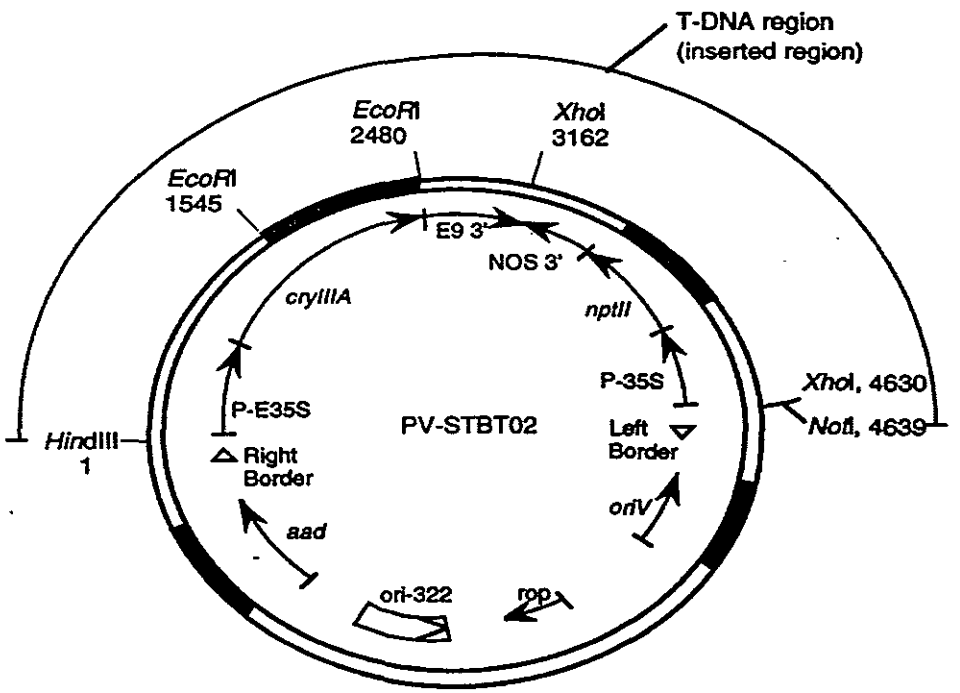

Figure III.1. Plasmid map of PV-STBT02.
Restriction sites, and their locations in base pairs, utilized during Southern analyses are shown. The region which served as the T-DNA is marked and its delineating right and lei borders are denoted by open arrows. The blackened regions denote the positions of homology for PCR probes used during Southern analyses as described in Section V.A. Cleavage sites for *Hin*dIII, *Eco*RI, *Xho*I and *Not*I restriction endonucleases are shown. A description of the genetic elements appears in Table III.1.

98

Canada and United States
Bilateral on Agricultural Biotechnology

Reviewers' Checklists

The Canadian Food Inspection Agency, Health Canada, and the United States Department of Agriculture's Animal and Plant Health Inspection Service, have prepared a series of checklists to be used by reviewers in the assessment process for the following six analytical techniques: Southern blot, Western blot, Northern blot, polymerase chain reaction, RNA dot blot, enzyme- linked immunosorbent assay (ELISA), and enzyme assays. The agencies are sharing these reviewers' checklists with potential applicants to provide guidance on the preparation of quality data.

Checklist for Northern Blot Data	YES	NO
Does the Northern blot have a figure number and title?		
Are lanes labeled on the blot?		
Does the figure legend describe each lane of the blot, including a description of the following for the RNA that was loaded: i. What type of material was loaded (eg. total purified RNA, poly-A RNA, crude prep, total plant extract)? ii. Source of the material loaded (eg. transformation event, tissue, developmental stage, any prior treatments to induce gene expression, etc.) iii. Quantity of material loaded in each lane?		
Does the text or figure legend describe how RNA was extracted prior to electrophoresis?		
Does the blot have appropriate positive and negative control lanes (positive control might demonstrate hybridization of the probe with itself; negative control might be the unmodified parental plant line or variety)?		
Is the gel system and Northern hybridization protocol described in the text or in a cited literature reference? Are any modifications of the cited protocols described in the petition text?		
Are the position of molecular size standards on the gel indicated, and do they cover an appropriate size range for the fragments that are expected to be detected on the blot?		
Is there a description of the probe that was used for the hybridization? If so, is the description adequate (in the text or in a figure) to enable one to interpret the results?		
If quantitative analysis is performed, has the methodology or citation to such been provided, and have a sufficient number of replicates or samples been tested to determine whether there are significant differences between samples or treatments?		
Are any superfluous bands or background signals properly explained?		

Checklist for Southern Blot Data	YES	NO
Does the Southern blot have a figure number and title?		
Are lanes labeled on the blot?		
Does the figure legend describe each lane of the blot, including a description of the following for the DNA that was loaded on the gel: i. Type of DNA loaded (eg. entire plasmid, restriction fragment)? ii. Source of DNA loaded (eg. transformation event, tissue)? iii. Restriction digestions of DNA prior to loading gel? iv. Quantity of material loaded in each lane?		
Does the gel have appropriate positive and negative control lanes (positive control might demonstrate hybridization of the probe with itself; negative control might be the unmodified parental plant line or variety)?		
Is the gel system and Southern hybridization protocol described in the text or in a cited literature reference? Are any modifications of the cited protocols described in the petition text?		
Are the position of molecular size standards indicated, and do they cover an appropriate size range for the fragments that are expected to be detected on the blot?		
Was an entire plasmid used as the probe for the hybridization? If so, is the plasmid described adequately in the text or in a figure to enable one to interpret the results?		
Was a restriction fragment used as the probe for the hybridization? If so, is the restriction fragment described adequately in the text or in a figure to enable one to interpret the results?		
Are any superfluous bands or background signals properly explained?		

Checklist for RNA Dot Blot Data	YES	NO
Does the dot blot have a figure number and title?		
Are lanes labeled on the blot?		
Does the figure legend describe each lane of the blot, including a description of the following for the RNA that was loaded: i. What type of material was loaded (eg. total purified RNA, poly-A RNA, crude prep, total plant extract) ii. Source of the material loaded (e.g., transformation event, tissue, developmental stage, any prior treatments to induce gene expression, etc.) iii. Quantity of material loaded in each lane?		
Does the text or figure legend describe how RNA was extracted prior to blotting onto the solid support?		
Does the blot have appropriate positive and negative control lanes (positive control might demonstrate hybridization of the probe with itself; negative control might be the unmodified parental plant line or variety)?		
Are the dot blot system and hybridization protocols described in the text or in a cited literature reference? Are any modifications of the cited protocols described in the submitted text?		
Is there a description of the probe that was used for the hybridization? If so, is the description adequate (in the text or in a figure) to enable one to interpret the results?		
If quantitative analysis is performed, has the methodology or citation to such been provided, and have a sufficient number of replicates or samples been tested to determine whether there are significant differences between samples or treatments?		

Checklist for Western Blot Data	YES	NO
Does the blot have a figure number and title?		
Are the lanes clearly labeled?		
Does the figure legend describe each lane of the blot, including a description of the following for the protein that was loaded: i. Type of material loaded (eg. crude, pure, total extract)? ii. Source of material loaded (eg. Transformation event, tissue type)? iii. Quantity of material loaded?		
Is the protein extraction method adequately described in either the text or the figure?		
Is the antibody or antiserum preparation protocol adequately described in the text, including an adequate description of the antigen and its purity? Has the specificity of antibody or antiserum been determined and described in the text or in a cited literature reference?		
Is the gel system and blotting protocol adequately described in the text or in a cited literature reference?		
Are the position of molecular weight standards indicated, and do they cover the appropriate range for the proteins expected to be detected on the blot?		
Does the blot include appropriate positive and negative controls?		
Was a normal serum control conducted?		
Are any superfluous bands or background signal properly explained?		
If quantitative analysis is performed, has the methodology or citation to such been provided, and have a sufficient number of replicates or samples been tested to determine whether there are significant differences between samples or treatments?		

Checklist for PCR Data	YES	NO
Does the PCR gel have a figure number and title?		
Are lanes labeled on the gel?		
Does the figure legend describe each lane of the gel, including a description of the following for the DNA that was loaded: i. What type of material was loaded (eg. plasmid fragment, amplified DNA)? ii. Source of material used in each reaction loaded (eg. transformation event, tissue)? iii. Quantity of material loaded?		
Are the position of molecular weight standards indicated, and do they cover an appropriate size range for the fragments that are expected to be detected on gel?		
Does the text or figure legend describe how PCR amplification was performed prior to electrophoresis?		
Is there a description of the primers used for amplification in the text or in the figure sufficient to enable one to interpret the results?		
Does the gel have appropriate positive and negative control lanes (positive control might demonstrate specificity of the primers and the ability to amplify the appropriate size band; negative controls might include amplification with DNA from the unmodified parental plant line or variety, and amplification in absence of DNA template)?		
Was an entire plasmid or a restriction fragment used as the positive control template and is it adequately described in the text or in the figure legend for interpretation of the PCR results?		
Is the gel system and PCR protocol described in the text or in a cited literature reference? Are modifications of a cited protocol described in the text?		

Checklist for ELISA Data	YES	NO
Does the table have a number and a title?		
Are all entries clearly identified in the table and described in the text or table legend?		
Is the sample preparation method described?		
Is the antibody or antiserum preparation protocol adequately described in the text, including a description of the antigen and its purity? Has the specificity of antibody or antiserum been demonstrated and described in the text or in a cited literature reference?		
Is the ELISA protocol used described in the text or cited in the scientific literature? Any modifications to a cited protocol must be described.		
Were appropriate positive controls (e.g. purified protein) and negative controls (e.g. normal or preimmune serum, non-transformed plant material) used?		
When ELISA is being used to quantify protein expression in transformed plant tissues: i. Was a method for the determination of protein concentration in tissue samples presented in the text or in a cited literature reference? ii. Was a standard curve prepared and the limit of detection indicated? iii. Have a sufficient number of replicates or samples been tested to determine whether there are significant differences between samples or treatments? Was statistical analysis performed?		

Checklist for Enzyme Assays	YES	NO
Does the figure (or table) have a number and title?		
For graphical representations or tables, are the axis or columns labeled and the units indicated?		
Does the scale of the figure accurately represent and allow interpretation of the data?		
Does the legend or text describe: i. The substrate and amount used for the reaction? ii. The quantity and origin of the enzyme? iii. The temperature and pH?		
Does the text or legend describe the extraction and purification of the enzyme and the degree of purification achieved?		
If the enzyme used in the assay has not been isolated from the transformed plant but is derived from an expression system, has adequate data been presented to demonstrate its substantial equivalence to the plant expressed enzyme?		
Have the assay method and relevant information concerning the enzyme been provided in the text or in a cited literature reference?		
Are appropriate controls included in the assay?		
Has the stability of the enzyme and possible presence of enzyme inhibitors in different tissue extracts been taken into account in the design of the assay or the interpretation of the data?		
When relevant to the safety assessment, have the kinetics of the enzyme been calculated and where possible compared to published data?		
When quantitative analysis is performed, have a sufficient number of replicates or samples been tested to determine whether there are significant differences between samples or treatments? Was statistical analysis performed?		

Annex 3

Powerpoint Presentation

at Workshop of Experts Gathered at AGERI

on June 20, 2001

Consultancy on Environmental Safety Assessment for Biotechnology in Egypt

USAID/APRP/RDI

Questions

- **Identify current environmental safety policies, procedures and practices related to the introduction of genetically engineered plants in Egypt.**
- **Determine how genetically engineered plants would be regulated under the current system.**
- **Develop a report describing options for the development and implementation of an evironmental safety regulatory plan for the commercialization of genetically engineered plants. Address laboratory qualifications to conduct assessments.**

Questions

- **Draft guidelines for the environmental assessment of genetically engineered plants.**
- **Draft a document describing how the environmental safety draft guidelines would be used to assess the potato variety "Spunta", genetically engineered to be resistant to potato tuber moth.**

Proposed Regulation

- The information submitted for assessment of food safety should be consistent with the guidelines set forth in Appendix VIII.
- The information submitted for assessment of environmental safety shall be consistent with the guidelines set forth in Appendix IX.

Proposed Appendix IX to the Biosafety Regulations and Guidelines

Guidelines for the environmental safety assessment of foods derived from genetically engineered plants

Principles

- Egypt is a signatory of the CBD and the Cartagena Biosafety Protocol
- Guidelines should be consistent with those adopted internationally, but applicable to Egypt.
- Guidelines are broad and flexible because cases will differ due to a wider range of variables to consider.

Principles

- More resposibility on the National Biosafety Committee to decide between "shades of gray". Identification of individual risk factors need not mean automatic rejection.
  - Safety is judged relative to the current level of risk, not absolute safety.
  - Risk management is an option for most regulatory schemes (e.g. Cartagena Protocol and E.U. guidelines)
  - Monitoring is an option for most regulatory schemes (e.g. Cartagena Protocol and E.U. guidelines)

Principles (Cartagena)

- **Objective**

  The objective of risk assessment, under this Protocol, is to identify and evaluate the potential adverse effects of living modified organisms on the conservation and sustainable use of biological diversity in the likely potential receiving environment, taking also into account risks to human health.

Principles (Cartagena)

- Use of risk assessment

  Risk assessment is, inter alia, used by competent authorities to make informed decisions regarding living modified organisms.

Principles (Cartagena)

- General principles
- Risk assessment should be carried out in a scientifically sound and transparent manner, and can take into account expert advice of, and guidelines developed by, relevant international organizations.
- Lack of scientific knowledge or scientific consensus should not necessarily be interpreted as indicating a particular level of risk, an absence of risk, or an acceptable risk.

Principles (Cartagena)

- Risks associated with living modified organisms or products thereof…should be considered in the context of the risks posed by the non-modified recipients or parental organisms in the likely potential receiving environment.
- Risk assessment should be carried out on a case-by-case basis. The required information may vary in nature and level of detail from case to case, depending on the living modified organism concerned, its intended use and the likely potential receiving environment.

Principles (Cartagena)

- Methodology
- The process of risk assessment may on the one hand give rise to a need for further information about specific subjects, which may be identified and requested during the assessment process, while on the other hand information on other subjects may not be relevant in some instances.

Principles (Cartagena)

- To fulfil its objective, risk assessment entails, as appropriate, the following steps:
  - An identification of any novel genotypic and phenotypic characteristics … that may have adverse effects on biological diversity in the likely potential receiving environment, taking also into account risks to human health;
  - An evaluation of the likelihood of these adverse effects being realized, taking into account the level and kind of exposure of the likely potential receiving environment to the living modified organism;

Principles (Cartagena)

- An evaluation of the consequences should these adverse effects be realized;
- An estimation of the overall risk posed by the living modified organism based on the evaluation of the likelihood and consequences of the identified adverse effects being realized;
- A recommendation as to whether or not the risks are acceptable or manageable, including, where necessary, identification of strategies to manage these risks; and
- Where there is uncertainty regarding the level of risk, it may be addressed by requesting further information on the specific issues of concern or by implementing appropriate risk management strategies and/or monitoring the living modified organism in the receiving environment.

Procedure for Egyptian Guidelines

- Used Cartagena Protocol Points to Consider as a starting point.
- Reviewed regulatory requirements for other countries, including the new E.U. draft regulations to replace Directive 90/220
- Synthesized the common requirements.

Environmental Safety Guidelines

I. Information on the recipient plant or parental plants

- Taxonomy:
  - family name;
  - genus;
  - species;
  - subspecies;
  - cultivar/breeding line;
  - common name.

I. Information on the recipient plant or parental plants

- Reproductive characteristics of the recipient plant
  - Information concerning reproduction:
    - » mode(s) of reproduction;
    - » specific factors affecting reproduction, if any;
    - » generation time
  - Sexual compatibility with other cultivated or wild plant species, including the distribution in Egypt of compatible species.

I. Information on the recipient plant or parental plants

- Survivability:
  - ability to form structures for survival or dormancy;
  - specific factors affecting survivability, if any.
- Allelopathy
- Dormancy

I. Information on the recipient plant or parental plants

- Dissemination (pollen, seed, vegetative means):
  - ways and extent (e.g. an estimation of how viable pollen and/or seeds declines with distance) of dissemination;
  - specific factors affecting dissemination, if any.
- Geographical distribution of the plant, including the centers of origin and genetic diversity, if known.

I. Information on the recipient plant or parental plants

- In the case of plant species not normally grown in Egypt, a description of the natural habitat of the plant, including information on natural predators, parasites, competitors and symbionts.
- Information on the location, geographical, climatic and ecological characteristics, including relevant information on biological diversity and centers of origin of the likely potential receiving environment

I. Information on the recipient plant or parental plants

- Other potential interactions of the plant with organisms in the ecosystem where it is usually grown, or elsewhere, including information on toxic effects on humans, animals and other organisms.

II. The sources of the new genetic materials (donor organisms and vectors, and their characterization in the genetically engineered plant

- Information on the donor organism(s) and, when appropriate, on other members closely related to the donor organism(s), including:
  - its scientific name
  - taxonomic classification
  - usual or common name.

- Information on the donor organism(s) and, when appropriate, on other members closely related to the donor organism(s), including:
  - its scientific name
  - taxonomic classification
  - usual or common name.

- Information on all genetic material potentially delivered to the engineered plant:
  - The specific method used for transformation.
  - The vector DNA, including the source (e.g. plant, microbial, viral , synthetic), identity and expected function in the plant.
  - Intermediate host organisms including the organisms (e.g. bacteria) used to produce or process DNA for transformation of the host organism
  - The DNA to be introduced (other than the vector), including:
    - » the characterization of all the genetic components including marker genes, regulatory and other elements affecting the function of the DNA
    - » the size and identity
    - » the location and orientation of the sequence in the final vector/construct
    - » the function

- Molecular and biochemical characterization of the DNA actually inserted into the plant genome, including
  - The number of insertion sites and location (e.g. chromosomal, chloroplast, mitochondrial
  - The organisation of the inserted genetic material at each insertion site, including sequence data of the inserted material and of the surrounding region if deemed necessary. Information should be presented regarding the arrangement of the genetic material used for transformation has been conserved or whether significant rearrangements have occurred upon integration.
  - The level and site of expression in the plant of the introduced gene product(s)
  - Data to demonstrate that all introduced traits are stably expressed and inherited.

III. Information on the genetically engineered plant and its effect on the receiving environment.

Information relating to known and potential differences between the genetically modified plant and the recipient plant in:

- Plant pest risk characteristics
- Disease and pest susceptibilities
- Expression of the gene product
- New enzymes
- Changes to plant metabolism

Information relating to known and potential differences between the genetically modified plant and the recipient plant in:

- **Weediness of the genetically engineered plant, including**
  - information concerning reproduction:
    - » mode(s) of reproduction
    - » specific factors affecting reproduction, if any
    - » generation time
  - dissemination (pollen, seed, vegetative means)
    - » ways and extent (e.g. an estimation of how viable pollen seeds declines with distance) of dissemination
    - » specific factors affecting dissemination, if any
  - survivability.
  - ability to form structures for survival or dormancy
  - specific factors affecting survivability, if any
  - allelopathy
  - dormancy

Information relating to known and potential differences between the genetically modified plant and the recipient plant in:

- Impact on the weediness of any other plant with which it can interbreed

Information relating to known and potential differences between the genetically modified plant and the recipient plant in:

- Intended use and agricultural or cultivation practices, including where applicable,
  - altered geographic range of cultivation
  - altered habitat adaptation

Information relating to known and potential differences between the genetically modified plant and the recipient plant in:

- **altered cultivation practices, including**
  - » information showing the effect of these changes on sustainability, especially with respect to pesticide use, frequency of tillage, soil erosion and consequential changes in energy and soil conservation
  - » information on any effect of volunteer plants on crop rotation practices
  - » specific containment or postharvest procedures, including procedures for disposal of remaining plant matter.
    - ■ any specified conditions for isolation as standard operating procedure for production
    - ■ any specified conditions for handling crop rotations as standard operating procedure for production
    - ■ any specified conditions for dealing with volunteers as standard operating procedure for production
  - » planting practices and policies (e.g. planting of mixtures to delay the evolution of resistance).

Information relating to known and potential differences between the genetically modified plant and the recipient plant in:

- ■ Effects of the genetically engineered plant on non-target organisms. If the introduced gene product is known to act by a toxic mechanism, the effects on potential non-target organisms that might be affected by such a mechanism should be evaluated, including soil micro flora and fauna.
- ■ Indirect plant pest effects on other agricultural products

Information relating to known and potential differences between the genetically modified plant and the recipient plant in:

- Transfer of genetic information to organisms with which it cannot interbreed
- Potential interaction with the abiotic environment.
- Description of detection and identification techniques for the genetically modified plant.
- Information about previous releases of the genetically modified plant, if applicable, including past reports provided to other regulatory agencies.

Note: Information required is gathered from a number of sources, not just tests. Reliance on the full range of information sources is necessary for a proper risk assessment.

Procedural Comments

- **Consultation with NBC early in the process is necessary to determine specific tests.**
  - Case by case requires understanding of tests to be done early as possible
  - Consultation provides for transparency of process

Procedural Comments

- Expertise should be drawn from appropriate disciplines, to comprise a working group with the necessary expertise to evaluate a specific application, for example
  - Plant breeder
  - Plant protection officer
  - Plant pathologist
  - Gene expression expert/molecular biologist
  - Health or food safety expert

Procedural Comments

- **While the guidelines have been presented as two separate entities, the final set produced by the NBC should be a unification of these, in order to produce a single dossier.**
- **Alternatively, a "User's Guide" to provide a model for safety assessment, incorporating the guidelines, can be produced.**

Annex 4

Preliminary Report on Existing Facilities and Human Resource Capacity for Assessment of Environmental Safety of Transgenic Plants in Egypt

Dr. Amin Sayed El-Nawawy

Introduction

Use of genetically modified organisms (GMOs) is currently subject to review and approval by the "National Biosafety Committee," which was established by a decree of the Minister of Agriculture and Land Reclamation. The work of the Committee is governed by the "Biosafety Regulations and Guidelines" established for this purpose.

The Committee is currently considering adopting specific protocols describing the environmental safety assessment that will be required for approval of GM plants for commercial release. It will be up to applicants to follow these protocols to complete a risk assessment for submission to the National Biosafety Committee for review. The design and conduct of the assessment should normally be done in consultation with the Committee. Much of the necessary data for a risk assessment can be drawn from scientific literature. Some of this literature, such as lists of wild plant species, is available through the biodiversity database of the Egyptian Environmental Affairs Agency (EEAA).

Actual tests in the field or laboratory may be conducted by the applicant itself or by government or private laboratories that are deemed qualified and contracted by the applicant for specific tests. In some cases, the Committee may ask an applicant to have a certain test done (or re-done) at a specific laboratory or institute. The Committee may draw on these same institutes to provide assistance in the review of applications.

There are several institutes and laboratories in Egypt that are capable of conducting tests necessary to meet the data requirements of the proposed "Guidelines for the Environmental Safety Assessment of Genetically Engineered Plants." Some of the best qualified institutes are in the government's Agricultural Research Center (ARC). Six of these institutes are discussed below in relation to their abilities to assist in an environmental risk assessment. Additional capacity is located in the government's National Research Center (NRC) and in several Egyptian universities.

It should be noted that this discussion is limited to environmental risk assessment of GM plants. Performance evaluation testing is done separately under the direction of the Variety Release Committee, after a variety is judged to be safe by the National Biosafety Committee.

1- Field Crop Research Institute (FCRI) in the Agricultural Research Center

The main objectives of FCRI is to develop, release and maintain high yielding cultivars and strains that are resistant to diseases, insects and tolerant to stress conditions, seeking to maximize crop productivity. The FRCI consists of 16 research departments. These include the breeding departments that work to improve cereal crops, legume crops, oil crops, forage crops, onion crop, and fiber crops. In addition, there are departments of weed control, seed technology and preservation, cell study, crop intensification, genetic resources and crop physiology.

The department of weed control is qualified to assist in Section III.F and Section III.G of the draft Guidelines for the Environmental Safety Assessment of Genetically Engineered Plants. These Sections address the weediness of the genetically engineered plant, including information on reproduction, dissemination, survivability, alleopathhy, and dormancy.

FCRI is also qualified to assist in Section III.H of the draft Guidelines, pertaining to intended use and cultivation practices. Field observations may be needed to determine if the applicant variety will require its user to plant, harvest, or employ post-harvest practices that are different from those of its non-genetically engineered equivalent. Particular attention should be put on proposed conditions, if any, for managing the evolution of resistance to any pest control traits.

2- Plant Protection Research Institute (PPRI) in the Agricultural Research Center

The main objectives of this institute include the identification, classification and survey of various crop pests of economic importance in Egypt. Such information is utilized in designing and implementing an Integrated Pest Management Program (IPMP). The PPRI also participates in the agricultural quarantine system to protect national agricultural resources against exotic pests that might be brought into Egypt through imported agricultural and food commodities. The PPRI also evaluates the efficiency of biological agents (fungi, bacteria, nematodes) involved in IPMP programs. The PPRI manages an insect collection unit that holds samples of foreign species.

The PPRI is qualified to assist in Section III.B of the draft Guidelines for the Environmental Safety Assessment of Genetically Engineered Plants. This Section addresses the pest susceptibilities of the GM plant. Differences in susceptibility to pests normally occurring in Egypt should be assessed, preferably through natural infestations and statistically valid experiments. The PPRI is also qualified to assist in Section III.I to assess effects on non-target organisms. If the introduced gene product is known to act by a toxic mechanism, the effects on potential non-target organisms should be evaluated. PPRI could, for example, conduct comparisons between insect species found on transgenic lines and non-genetically engineered equivalent lines.

3- Plant Pathology Research Institute (PPathRI) in the Agricultural Research Center

The main objective of this institute is to protect plant crops in Egypt from different diseases that affect crop production, causing national losses. Extensive research, extension, and applied activities are being carried out in this institute. PPathRI surveys and identifies plant disease pathogens (fungi, bacteria, virus) as well as evaluating fungicides, nematocides, bioagents, and plant extracts that can control diseases of different crops. Rapid detection of virus using DNA hybridization, PCR and ELIZA is being applied in certain labs of this institute. Research staff in the integrated control department are familiar with isolation and identification of the microbial population grown on wild and cultivated varieties as well as GM plants. Besides they have experience in detection of toxin produced in infected plants. PPathRI has a DNA finger print lab, where staff are able to identify and register different GMOs, according to their DNA structure.

The institute includes separate departments for each crop diseases, where they can study susceptibility or resistance of applicant GMOs as compared with conventional varieties. The PPathRI is qualified to check the information provided by the applicant in relation to Section II.B of the proposed Guidelines. This includes review of the vector DNA, the map of the vector plasmid and the characterization of all of the genetic components including marker genes.

The PPathRI is also qualified to assist in Section III.B of the draft Guidelines for the Environmental Safety Assessment of Genetically Engineered Plants. This Section addresses the disease susceptibilities of the GM plant. Differences in susceptibility to disease normally occurring in Egypt should be assessed, preferably through natural infestations and statistically valid experiments. The PPathRI is also qualified to assist in Section III.C to assess expression of the gene product and Section III.D to assess new enzymes.

4- Soils, Water & Environment Research Institute (SWERI) in the Agricultural Research Center

SWERI conducts basic and applied research on soil, water and plant inter-relationships, soil survey and classification, soil amelioration, plant nutrition and water requirements. There are separate departments for agricultural microbiology, which deals mainly with microbial activity in soil and water; bioconversion systems for agricultural residues for production of food, feed, organic fertilizers and bioenergy; bioremediation; biocontrol of nematodes, fermentation; role of plants and microbes on the activity of rhizosphere. The agricultural microbial department can contribute in detecting the effects of GMOs on microbial activity in the rhizosphere; exudates of plant roots of GMOs and their effect on soil microflora; following, detecting, adjustment of procedures and treatments for disposable remaining GMOs in soil and/or in atmosphere.

SWERI is qualified to assist in Section III.I of the Guidelines to assess effects on non-

target organisms. If the introduced gene product is known to act by a toxic mechanism, the effects on potential non-target organisms should be evaluated. The agricultural microbial department of SWERI could, for example, conduct comparisons between a census of soil micro flora and fauna under the GM plant and micro flora and fauna under non-genetically engineered equivalent lines.

5- Horticulture Research Institute (HRI) in the Agricultural Research Center

The major goal of HRI is to promote productivity of the horticultural crops. HRI improves varieties by importing foreign varieties and/or developing local varieties and hybrids of high yield and superior quality. The institute includes in his program the breeding and development of new improved varieties of higher yields and superior quality, by using genetic engineering techniques. Also the institute planned to identify fingerprints of horticulture crops by using new biotechnology techniques. There is cooperation between HRI and AGERI.

HRI is qualified to assist in Section III.H of the draft Guidelines, pertaining to intended use and cultivation practices. Field observations may be needed to determine if the applicant variety will require its user to plant, harvest, or employ post-harvest practices that are different from those of its non-genetically engineered equivalent. Particular attention should be put on proposed conditions, if any, for managing the evolution of resistance to any pest control traits.

6- Agricultural Genetic Engineering Research Institute (AGERI) in the Agricultural Research Center

This institute is the best equipped and qualified to carry out most of the tests needed for an environmental risk assessment. However, because AGERI is a producer of transgenic plant varieties, conflicts of interest may arise if it is also involved in testing for regulatory purposes. In any case, AGERI is capable of generating much of the risk assessment data required for its own applications to the NBC. And, in certain cases, the institute could be used by other applicants or by the NBC itself to develop or check data in other applications. This would need to be decided on a case by case basis and in light of the availability of alternative sources for testing data.

AGERI has capacity for advanced molecular biology and genetic engineering. AGERI is the official body of the Ministry of Agriculture for GMO R&D . Its projects include participation from university staff members, including a diverse multi-disciplinary group of plant pathologists, breeders, microbiologists and horticulturists who are joint appointees.

AGERI is involved in a wide range of projects from developments of Bt strains as biopesticides to development of transgenic plants. Research includes engineering plants for biotic and abiotic stresses, including viral, fungal, insect and bacterial resistance; and salinity, heat and drought resistance. Out of these projects, there are

two genetically engineered crops now approaching the stage of commercial release (potato engineered to resist infestation by potato tuber moth, and squash plants resistant to major viral pathogens).

At AGREI, there are ten functional laboratories: molecular plant pathology, molecular manipulation and gene transfer, plant molecular biology, molecular genetics and genome mapping, micropropagation technology, plant cellular and molecular genetics, immunology and diagnostics, nucleic acid research and protein structure, gene expression, and biocomputing and network unit.

AGERI is qualified to develop and/or check the information provided by the applicant in relation to Section II.B and II.C of the proposed Guidelines. This includes review of the vector DNA, the map of the vector plasmid and the characterization of all of the genetic components including marker genes. Section II.C includes molecular and biochemical characterization of the DNA actually inserted into the plant genome. Such characterization could by provided through a Southern blot analysis in many cases.

AGERI is also qualified to assist in Section III.C to assess expression of the gene product and Section III.D to assess new enzymes.

7- National Research Center (NRC)

A division of genetic engineering and biotechnology was established with six departments: Molecular biology, cell biology, plant cell and tissue culture, microbial biotechnology, microbial genetic and human department. The main activities of the first five departments are:

- ***Molecular biology***: for enzyme production for industrial and clinical diagnosis.
- ***Cell biology:*** for animal cytogenetics (chromosome and gene mapping) biotechnological methods for embryo technology (embryo culture, transfer, sexing, splitting pre-implantation, embryo freezing and twinning)
- ***Plant cell and tissue culture:*** for plat tissue culture to produce secondary metabolites: the selection of plant cell lines for stress tolerance e.g. to salinity and drought: the production of virus – free potato planting material: and the micropropagation of plants with difficulties for conventional propagation.
- ***Microbial biotechnology***: for ethanol production from sugar by – products and methane production from agro-industrial wastes.
- ***Microbial genetics:*** for the production of useful microbial compounds through genetically improved strains such as saponin production in *Poinciana regia* to *Azotobacter;* elimination or degradation of pollutants; transformation of

celluloytic nitrogen fixers; construction of *Saccharomyces cerevisiae* strains capable of cellulose, cellobiose or lactose consumption.

8- Genetic Engineering and Biotechnology Research Institute: (GEBRI):

This institute is part of the Mubarak City Science and Technology center in Alexandria. One of its main goals is to contribute to the promotion of biotechnology R&D in the areas of health, environmental, bioprocessing, marine biotechnology and agricultural biotechnology. GEBRI strives to improve and develop knowledge and expertise in biotechnology and genetic engineering. The work of GEBRI is concentrated in five main programs:

- Proteins Research Program;
- Nucleic Acid Research Program (gene mapping and modification, production of DNA probes and primers for use in PCR–based diagnostic kits),
- Biochemical Research Program (devoted to health related biotechnology), and
- Bioremiation Research program; and
- Biotechnology Product Development Program, which functions as a pilot facility for the production of recombinant gene products.

9- Universities

There are 13 large universities that support science faculty. Each has departments covering most scientific fields, including medicine, pharmacology, veterinary medicine, engineering, agriculture and dentistry. Egypt's university system has a new awareness of generic engineering and biotechnology and its potential. Many academic scientists with strong abilities, education and experience are currently working in Egyptian universities and institutes. However, lack of funding and equipment can hamper their ability to perform their researches.

Examples of research centers of excellence in Egyptian Universities are:

- Research Center of Genetic Engineering: Faculty of Agriculture, Cairo University. Main activities include tissue culture, recombinant DNA, genetic transfer, gene cloning for pest control, recombinant DNA, genetic transfer, gene cloning for pest control and environmental stress, and biofertilizers.

- Biotechnology Research Center – Faculty of Pharmacy Cairo University. Activities are mainly in industrial biotechnology, bioremediation, molecular biology, and tissue culture.

- Ain Shams Center for Genetic Engineering and Biotechnology (ACGEB). Main activities include identification of plant genetic resource – molecular genetics,

gene cloning and mutagenic potential of pesticides on cellular and subcellular levels, DNA finger–prints.

- Microbiological Resource Center (MIRCEN) – Faculty of Agriculture – Ain Shams University. It represents part of the international program for stimulating the conservation and utilization of microbial genetic resources for environmental management in developing countries.

- Genetic Engineering and Biotechnology Institute- Faculty of Agriculture, Menofya University – Sadat City. The institute involved in tissue culture production, genetic transfer, gene cloning, and molecular biology.

- Genetic Engineering and Biotechnology Lab – Faculty of Agriculture – Alexandria University - Training for several researchers in G.E. and biotechnology. There is also a strong research program in plant pathology and gene transfer.

- Suez Canal Center for Biotechnology, which is mainly interested in microbial biotechnology.

- Genetic Engineering and Molecular Biology Research Center. Assuit University – has diagnostic activities.

- Institute for Environment Studies and Research (IESI), Ain Shams University.

- Institute for Graduate Studies and Research, Alexandria University.

Annex 5

Current Decrees and Laws with Impact upon the Commercial Release of Transgenic Plants

Prof. Dr. Amin Sayed El-Nawawy

During the period 1994-99, a number of Ministerial Decrees were issued to accommodate plant breeders and/or private sector rights and obligations for releasing GM plants.

(1) Decree No. 242/1997, Ministry of Health: This decree prohibits importing any foodstuff produced through GMOs, unless its safety is confirmed. The act also necessitates that a certificate should accompany any imported seeds from the country of origin, confirming that these seeds were not produced from untested genetically modified (GM) plants, GM plants or seeds can be imported if previously approved for use in the country of origin and deemed safe.

(2) Decree No. 821/1998, Ministry of Agriculture: This decree establishes policy and provides guidance on procedures for the release of crop varieties developed by the ARC. It makes no distinction between varieties developed through conventional breeding and those derived by genetic engineering. Variety identification or Distinctness, Uniformity and Stability (DUS) tests are conducted in one location during seed multiplication. Model descriptors issued by the International Union for the Protection of New Varieties of Plants (UPOV) are used for a comparative evaluation against standard varieties as defined by the relevant Crop Seed technical committee, which are grown in parallel with the new variety. Variety performance or Value of Cultivation and Use (VUC) tests examine the new variety's agricultural, industrial, and economic value as compared with other superior varieties in use. Such tests are conducted in more than one location to ensure that the variety tolerates a diversity of local environment conditions. The candidate variety is recommended to be registered only when all required tests have been satisfactorily completed.

(3) Decree No. 1648/1998, Ministry of Agriculture: This decree confirms the authority and responsibility of CASC for releasing GM as well as conventional seeds. It describes procedures for obtaining a small-scale release permit for a new genetically engineered crop variety, registering it, and releasing it for commercial use. It outlines important steps to be followed by government or private sector applicants, as well as other local or foreign organizations seeking to commercialize their products. The decree specifies the roles and responsibilities of the NBC, the Seed Registration Committee, and the Committee for Food Safety. A draft paper outlining the protocol for commercial release of GMO crop varieties

was developed by a panel of experts from MALR and the USAID. The final document was approved by deputy Prime Minister and Minister of Agriculture in July 1999 .

(4) Decree No. 702/1999, Ministry of Agriculture: This decree adds DNA fingerprinting to the required protocol for registration of all new agriculture crop varieties in order to confirm identity during the registration process and for subsequent use as a reference, if required. The decree stipulates that:

- DNA fingerprints of the new hybrid variety and its parents are a prerequisite for registration. One copy of the fingerprint is to be kept in the secretariat of the Seed Registration Committee and another copy is to be kept in the management office of the applicant's institution
- The relevant crop technical committee should verify the fingerprint and its specifics through a laboratory certified to have the required scientific and technical capabilities.
- The applicant is to pay all costs required for the finger printing process, as determined by the registration committee for agricultural varieties. Sample material is to be submitted to the SRC secretariat, which will pass it to the relevant certified lab.

(5) Law 4 of 1994 is by far the most comprehensive environmental legislation to date. It defines (in article 2-13) the scope and responsibilities of the Egyptian Environmental Affairs Agency (EEAA), establishes (in article 14-16) the Environmental Protection Fund (EPF), provides for the setting up of a system of environmental incentives (articles 17-18), spells out the necessity of environmental impact assessments as a pre-requisite of development projects (article 19-23), establishes environmental monitoring networks with their stations and working units (article 24), authorizes the EEAA to prepare an Environmental Contingency Plan (article 25), and forbids the hunting of specified types of wild birds and animals (article 28) as well as the destruction of their natural habitats. Articles 29-83 cover the protection of air, water and land from all sources of pollution. Articles 84-101 deal with the penal code for violation of articles 1-83. In August 1997 the EEAA became part of the newly established Ministry of State for Environmental Affairs. This law does not mention any direct statement on GMOs. However, the NBC is in contact with the Ministry of Environment for the preparation of appropriate regulations under a law and not only as a ministerial decree.

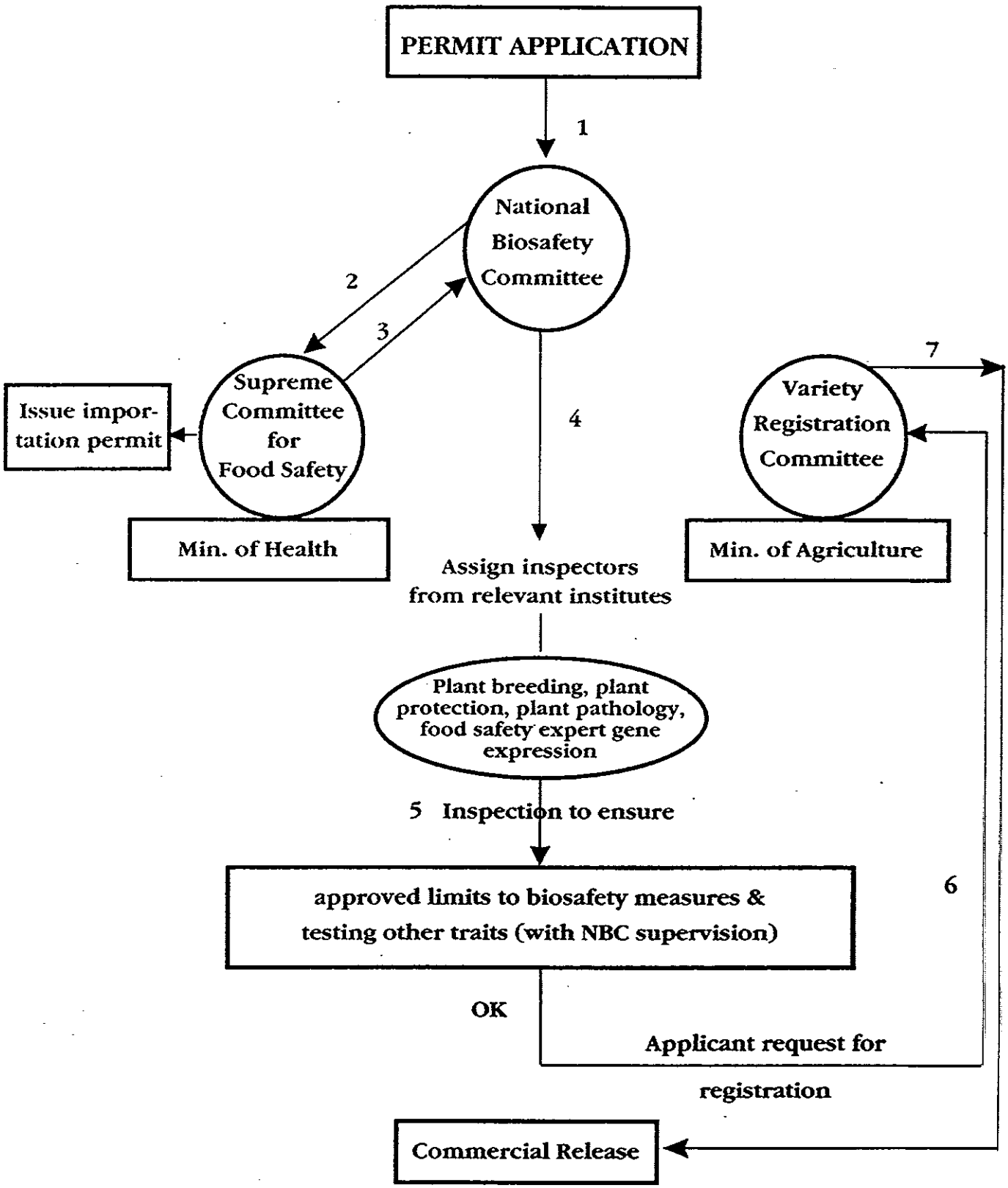

Chart I: Steps for Commercial Release of Genetically Engineered Plants in Egypt